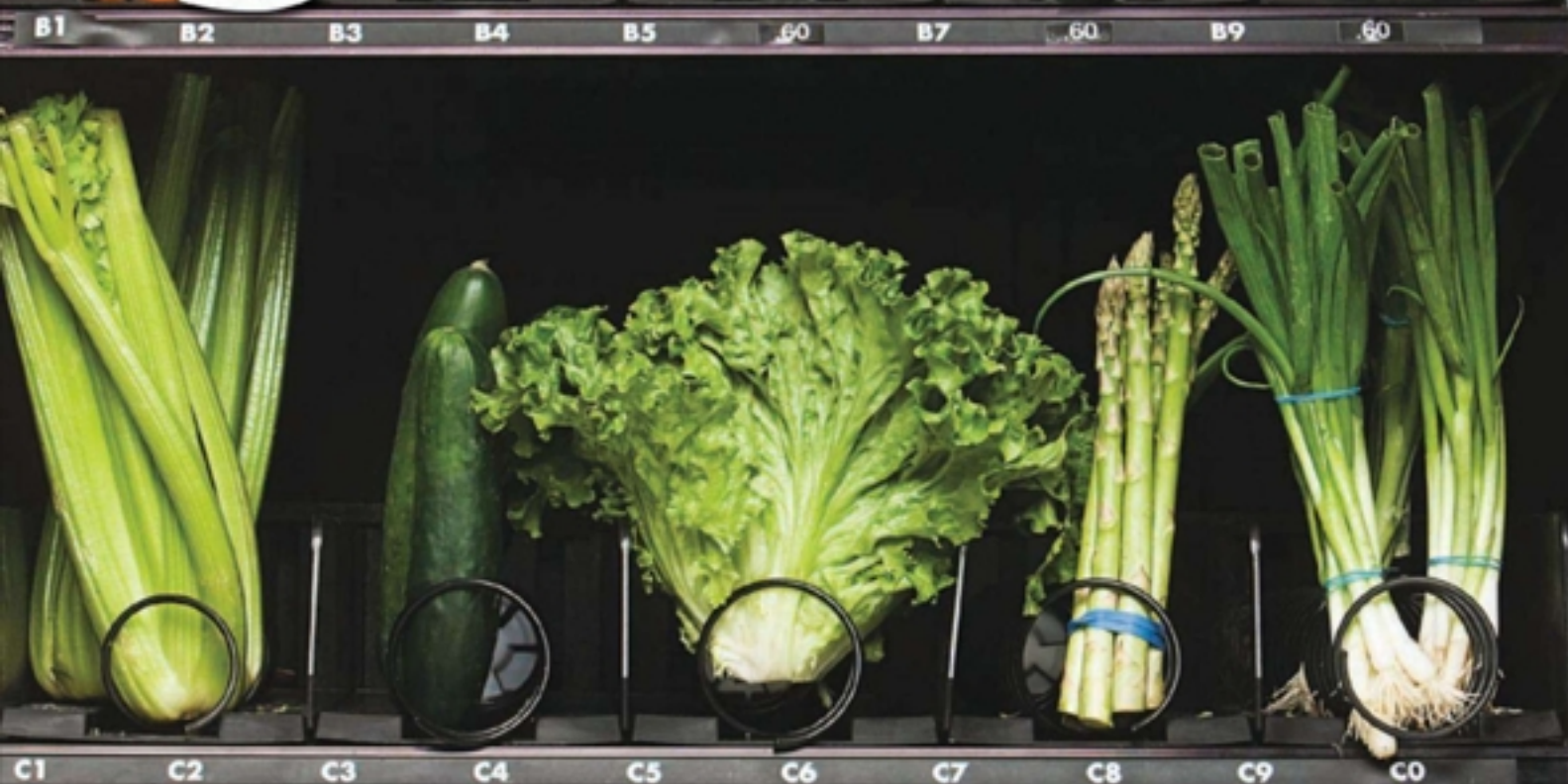


# Science

21 September 2012 | \$10



## DISEASE PREVENTION



AAAS

## SPECIAL SECTION

# Disease Prevention

### INTRODUCTION

- 1466** It Takes More Than an Apple a Day

### NEWS

- 1468** Task Force's Prevention Advice Proves Hard to Swallow  
Public Health Measures in Disease Prevention  
>> *Science Podcast*
- 1471** Will an Aspirin a Day Keep Cancer Away?  
Wondering How the Wonder Drug Works
- 1473** Tackling America's Eating Habits, One Store at a Time  
>> *Science Podcast*
- 1476** Uncertain Verdict as Vitamin D Goes on Trial
- 1479** Chronic Disease Vaccines Need Shot in the Arm

### REVIEW

- 1482** Can Noncommunicable Diseases Be Prevented? Lessons from Studies of Populations and Individuals  
*M. Ezzati and E. Riboli*

### PERSPECTIVES

- 1488** Preventing Alzheimer's Disease  
*D. J. Selkoe*  
>> *Science Podcast*
- 1492** Changing Human Behavior to Prevent Disease: The Importance of Targeting Automatic Processes  
*T. M. Marteau et al.*
- 1495** Fetal and Early Childhood Undernutrition, Mortality, and Lifelong Health  
*C. K. Lutter and R. Lutter*
- 1499** Double Burden of Noncommunicable and Infectious Diseases in Developing Countries  
*I. C. Bygbjerg*
- 1501** Why a Macroeconomic Perspective Is Critical to the Prevention of Noncommunicable Disease  
*R. Smith*  
>> *Science Podcast*

>> *Editorial p. 1433; Policy Forum p. 1456; Science Careers; and videos at [www.sciencemag.org/special/prevention](http://www.sciencemag.org/special/prevention)*

### EDITORIAL

- 1433** Prevention and Cost Control  
*Ezekiel Emanuel*  
>> *Disease Prevention section p. 1466*

### NEWS OF THE WEEK

- 1438** A roundup of the week's top stories

### NEWS & ANALYSIS

- 1441** New XMRV Studies Bring Closure—and Fresh Dispute
- 1442** Warped Light Reveals Infant Galaxy on the Brink of the 'Cosmic Dawn'
- 1443** Did Neandertals Truly Bury Their Dead?
- 1444** Ignition Facility Misses Goal, Ponders New Course

- 1446** An Enterprising Time for HIV Vaccine Research
- 1447** Cancers Join List of Illnesses Linked to 9/11 Attacks

### NEWS FOCUS

- 1448** China's Sharp Focus on Biometrics
- 1450** Despite Setbacks, Optimism on Drugs for Hepatitis C

### LETTERS

- 1452** Friends in Fungi  
*G. D. A. Werner and E. T. Kiers*
- Political Science in Peril  
*A. Lupia*
- 1453** The BUZZ: Honorary Authorship
- 1453** TECHNICAL COMMENT ABSTRACTS
- 1453** CORRECTIONS AND CLARIFICATIONS



page 1448

### BOOKS ET AL.

- 1454** Interop  
*J. Palfrey and U. Gasser, reviewed by L. DeNardis*
- 1455** Wild Hope  
*A. Balmford, reviewed by D. McCauley*

### POLICY FORUM

- 1456** Global Indicators and Targets for Noncommunicable Diseases  
*S. Y. Angell et al.*  
>> *Disease Prevention section p. 1466*

### PERSPECTIVES

- 1458** A Measurable Planetary Boundary for the Biosphere  
*S. W. Running*
- 1459** Nanometer-Scale Printing  
*J. A. Rogers*  
>> *Report p. 1517*
- 1460** Versatility from Protein Disorder  
*M. M. Babu et al.*
- 1462** Thermally Responsive Pulsating Nanotubules  
*W. Zhang and T. Aida*  
>> *Report p. 1521*
- 1463** Emerging Anatomy of the BAP1 Tumor Suppressor System  
*A. E. White and J. W. Harper*  
>> *Report p. 1541*
- 1464** Moving and the Neighborhood Glass Ceiling  
*R. J. Sampson*  
>> *Research Article p. 1505*

**CONTENTS** continued >>



### COVER

A vending machine filled with healthy substitutes for highly processed foods. Affluence paved the way for technology and medicines to combat infection, but even well-off nations have communities that are increasingly plagued by noncommunicable diseases, including cancer, type 2 diabetes, and Alzheimer's disease. Addressing this huge prospective burden of long-term disease is a priority in the design of a new generation of preventative strategies, including healthier eating. See our special section beginning on page 1466.

Image: Mike Kemp/Getty Images

### DEPARTMENTS

- 1431** This Week in *Science*
- 1434** Editors' Choice
- 1436** *Science* Staff
- 1557** New Products
- 1558** *Science* Careers



## RESEARCH ARTICLE

- 1505 Neighborhood Effects on the Long-Term Well-Being of Low-Income Adults**  
*J. Ludwig et al.*  
 Moving from a poor to a less poor residential environment makes people feel good for a long time.  
 >> *Perspective p. 1464*

## REPORTS

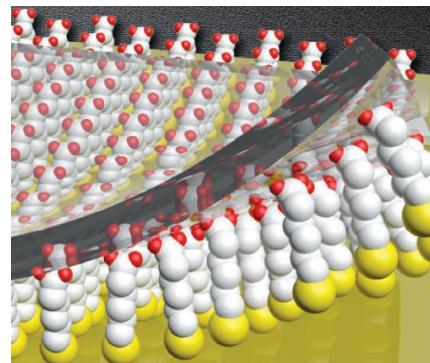
- 1511 Kepler-47: A Transiting Circumbinary Multiplanet System**  
*J. A. Orosz et al.*  
 Data from the Kepler space telescope reveal two small planets orbiting a pair of two low-mass stars.
- 1514 Quantum-Enhanced Optical-Phase Tracking**  
*H. Yonezawa et al.*  
 A quantum mechanical technique is developed to enhance the phase tracking of photons.
- 1517 Subtractive Patterning via Chemical Lift-Off Lithography**  
*W.-S. Liao et al.*  
 Oxidized silicone-rubber stamps can pattern bare regions on gold surfaces by removing hydroxyl-terminated alkane thiols.  
 >> *Perspective p. 1459*
- 1521 Pulsating Tubules from Noncovalent Macrocycles**  
*Z. Huang et al.*  
 Nanoscale tubes expand and contract with temperature changes through loose association of their constituent building blocks.  
 >> *Perspective p. 1462*
- 1526 A Crystalline Singlet Phosphononitrene: A Nitrogen Atom–Transfer Agent**  
*F. Dielmann et al.*  
 A phosphorus fragment supports an otherwise uncoordinated nitrogen atom in a stable motif previously seen only with metals.
- 1529 A Fully Size-Resolved Perspective on the Crystallization of Water Clusters**  
*C. C. Pradzynski et al.*  
 Infrared spectroscopy of water clusters of increasing size indicates the onset of an ice-like structure at around 275 molecules.

- 1532 Structural Basis for Microtubule Binding and Release by Dynein**  
*W. B. Redwine et al.*  
 The molecular motor dynein uses conformational changes within its microtubule-binding domain to modulate track affinity.
- 1536 Specifying and Sustaining Pigmentation Patterns in Domestic and Wild Cats**  
*C. B. Kaelin et al.*  
 The genes specifying tabby cat coat patterns also affect big cats, including king cheetahs.
- 1541 Loss of the Tumor Suppressor BAP1 Causes Myeloid Transformation**  
*A. Dey et al.*  
 The de-ubiquitinating enzyme BAP1 is implicated in myelodysplastic syndrome.  
 >> *Perspective p. 1463*
- 1546 Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga**  
*A. W. Thompson et al.*  
 A nitrogen-fixing cyanobacterium that lacks photosynthesis and the tricarboxylic acid cycle possesses a tiny phytoplankton symbiont.
- 1550 Disruption of Reconsolidation Erases a Fear Memory Trace in the Human Amygdala**  
*T. Agren et al.*  
 Interfering with memory formation at the right moment removes the signature of fear memories in human brain imaging studies.
- 1553 Acute Gastrointestinal Infection Induces Long-Lived Microbiota-Specific T Cell Responses**  
*T. W. Hand et al.*  
 Enteric infections induce lasting adaptive immunity against commensal bacteria that may play a role in intestinal problems.

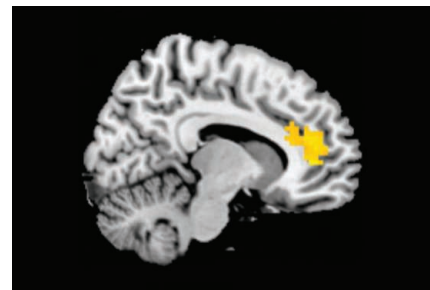
CONTENTS continued &gt;&gt;



page 1454



pages 1459 &amp; 1517



page 1550

## SCIENCEONLINE

## SCIENCEEXPRESS

[www.sciencexpres.org](http://www.sciencexpres.org)

### Elemental Mapping by Dawn Reveals Exogenic H in Vesta's Regolith

*T. H. Prettyman et al.*

10.1126/science.1225354

### Pitted Terrain on Vesta and Implications for the Presence of Volatiles

*B. W. Denevi et al.*

Analysis of data from the Dawn spacecraft implies that asteroid Vesta is rich in volatiles.

10.1126/science.1225374

### A Golden Spike for Planetary Science

*R. P. Binzel*

10.1126/science.1228328

### Detecting Causality in Complex Ecosystems

*G. Sugihara et al.*

A new method, based on nonlinear state space reconstruction, can distinguish causality from correlation.

10.1126/science.1227079

### Genomic Variation in Seven Khoe-San Groups Reveals Adaptation and Complex African History

*C. M. Schlebusch et al.*

Cutting-edge genomic approaches test hypotheses about the roots of human history in southern African indigenous populations.

10.1126/science.1227721

### Germ Cell Migration Across Sertoli Cell Tight Junctions

*B. E. Smith and R. E. Braun*

Syncytial germ cell chains span a network of transient compartments to cross the blood-testis barrier in adult mice.

10.1126/science.1219969

## TECHNICALCOMMENTS

### Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

*J. E. Cresswell and H. M. Thompson*

Full text at [www.sciencemag.org/cgi/content/full/337/6101/1453-b](http://www.sciencemag.org/cgi/content/full/337/6101/1453-b)

### Response to Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

*M. Henry et al.*

Full text at [www.sciencemag.org/cgi/content/full/337/6101/1453-c](http://www.sciencemag.org/cgi/content/full/337/6101/1453-c)

## SCIENCENOW

[www.sciencenow.org](http://www.sciencenow.org)

Highlights From Our Daily News Coverage

### Whodunit? Crows Ask That Question, Too

New Caledonian crows, like humans, can reason about hidden causes.

<http://scim.ag/Crows-Question>

### Plight of the Bumblebee

Did invader insects bring deadly disease to South American bees?

<http://scim.ag/Plight-Bumblebee>

### Early Isolation Impairs Brain Connections

Childhood neglect stunts the brain's 'insulating' cells.

[http://scim.ag/Insulating\\_Cells](http://scim.ag/Insulating_Cells)

## SCIENCE SIGNALING

[www.sciencesignaling.org](http://www.sciencesignaling.org)

The Signal Transduction Knowledge Environment

18 September issue: <http://scim.ag/ss091812>

### RESEARCH ARTICLE: Charting the Landscape of Tandem BRCT Domain-Mediated Protein Interactions

*N. T. Woods et al.*

Building a protein-protein interaction network mediated by the BRCT domain reveals players in the DNA damage response.

### PERSPECTIVE: Modulating Mitochondrial Intracellular Location as a Redox Signal

*M. P. Murphy*

Hypoxia induces the redistribution of mitochondria to promote transcriptional responses to low oxygen.

### REVIEW: Pseudogenes—Newly Discovered Players in Human Cancer

*L. Poliseno*

Pseudogenes can have multiple, diverse functions, affecting not only their parental genes but also unrelated genes.

## SCIENCE TRANSLATIONAL MEDICINE

[www.sciencetranslationalmedicine.org](http://www.sciencetranslationalmedicine.org)

Integrating Medicine and Science

19 September issue: <http://scim.ag/stm091912>

### RESEARCH ARTICLE: Effects of STX209 (Arbaclofen) on Neurobehavioral Function in Children and Adults with Fragile X Syndrome: A Randomized, Controlled, Phase 2 Trial

*E. M. Berry-Kravis et al.*

Administration of a selective GABA<sub>B</sub> agonist to individuals with fragile X syndrome improves their deficits in social avoidance, a core symptom of the disease.

### RESEARCH ARTICLE: Reversal of Disease-Related Pathologies in the Fragile X Mouse Model by Selective Activation of GABA<sub>B</sub> Receptors with Arbaclofen

*C. Henderson et al.*

Pharmacological activation of the GABA<sub>B</sub> receptor with arbaclofen in a mouse model of fragile X syndrome corrects neuronal defects associated with the disease.

### RESEARCH ARTICLE: A Paper-Based Multiplexed Transaminase Test for Low-Cost, Point-of-Care Liver Function Testing

*N. R. Pollock et al.*

A paper-based, multiplexed microfluidic assay allows rapid, semiquantitative, visual measurement of transaminases in clinical specimens.

### PERSPECTIVE: Patient-Empowerment Interactive Technologies

*C. S. Bruggers et al.*

Health-promoting video games are emerging as nonpharmacological tools to help patients manage their diseases.

### PERSPECTIVE: Childhood Disorders of the Synapse—Challenges and Opportunities

*V. Hughes et al.*

New research directions are needed to accelerate the development of new treatments for autism and related disorders.

## SCIENCE CAREERS

[www.sciencereers.org/career\\_magazine](http://www.sciencereers.org/career_magazine)

Free Career Resources for Scientists

### Documenting Exposure for Disease Prevention

*E. Pain*

Epidemiologist Emilie Council studies the health implications of environmental carcinogen exposure.

<http://scim.ag/EmilieCouncil>

>> *Disease Prevention* section p. 1466

### Tooling Up: What's Your Question?

*D. Jensen*

Never mind answering questions—what questions should you ask at a job interview?

<http://scim.ag/WhatsYourQuestion>

### myIDP: So You Think You Have Skills

*J. Hobin et al.*

Figuring out what you know—and what you need to know—is essential in training for a science career.

<http://scim.ag/myIDPSkills>

## SCIENCE PODCAST

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Free Weekly Show

On the 21 September *Science* Podcast: a special show on preventing noncommunicable disease, including the economics of prevention, government recommendations, combating Alzheimer's, and grocery studies.

## SCIENCE INSIDER

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Science Policy News and Analysis

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## << What Kitty Shares with Kings

Although long-studied, the underlying basis of mammalian coat patterns remains unclear. By studying a large number of cat species and varieties, **Kaelin *et al.*** (p. 1536) identified two genes, *Taqpep* and *Edn3*, as critical factors in the development of feline pigment patterns. Mutations in *Taqpep* are responsible for the blotched tabby pattern in domestic cats and the unusual coat of wild king cheetahs. Gene expression patterns in cat and cheetah skin suggest that *Edn3* is a likely regulator of felid hair color. The findings support a common model for coat and pigment pattern formation in domestic and wild cats.

## Location, Location, Location

It seems obvious that a person's residential neighborhood will influence their sense of well-being, but it has been difficult to nail down cause and effect. **Ludwig *et al.*** (p. 1505; see the Perspective by **Sampson**) describe the analysis, 10 to 15 years onward, of a large-scale social experiment carried out in five U.S. cities in the mid 1990s. Several thousand residents of poor neighborhoods were given housing vouchers that could only be used if they moved into much less poor neighborhoods. In comparison to a similar group of individuals who did not move, those who did experienced substantial improvement in their subjective well-being.

## N on P

Nitrogen atoms form strong, relatively unreactive triple bonds with themselves (in  $N_2$ ) and with carbon (in cyanide and nitriles). In contrast, binding to transition metals often leaves an otherwise naked nitrogen center more prone to reactivity. **Dielmann *et al.*** (p. 1526) prepared a compound with nitrogen bound to divalent phosphorus, which acted more like a metal than a light element. Although the compound, formally a nitrene, was sufficiently stable to isolate at room temperature and characterize by x-ray diffraction, it transferred the nitrogen efficiently to unsaturated carbon compounds.

## Minimal Ice

Water clusters comprising fewer than 100 molecules have long been studied in gas phase

to model the more complex structures of ice and liquid water. At some stage, as clusters grow larger, they effectively become tiny crystals of ice, but it has been hard to pinpoint precisely where in the range between 100 and 1000 molecules the formal transition takes place. **Pradzynski *et al.*** (p. 1529) used vibrational spectroscopy to show that the onset of an ice-like structure, indicated by a characteristically distinct absorption band in the infrared, occurs at a cluster size of approximately 275 molecules.

## Keeping Track of Photon Phase

In optical interferometers or optical communications, information is often stored in terms of the phase of the waveform or light pulse. However, fluctuations and noise can give rise to random jitter in the phase and amplitude of the optical pulses, making it difficult to keep track of the phase. **Yonezawa *et al.*** (p. 1514) developed a technique based on quantum mechanical squeezing to determine the phase of randomly varying optical waveforms. The quantum mechanical technique enhanced the precision with which the phase could be determined and, as optical technologies continue to be miniaturized, should be helpful in applications within metrology.

## Patterning by Subtraction

Soft lithographic patterning is usually a "positive" inking process. A polymer stamp is cured on a hard master substrate and then inked with molecules such as alkane thiols, which can then

be transferred to a second substrate (such as gold). However, the resolution of the transferred pattern is often degraded by surface diffusion. **Liao *et al.*** (p. 1517; see the Perspective by **Rogers**) obtained higher resolution in a subtractive approach, in which oxygen-plasma-activated silicone stamps removed hydroxyl-terminated alkane thiols from gold surfaces. This lift-off process also removed the terminal gold atom bound to the alkane thiol. The bare regions could be backfilled with protein molecules, and multiple lift-off steps could create patterns with features as small as 40 nanometers.

## Slip-Sliding Apart

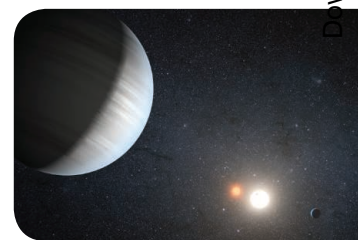
One versatile means of synthesizing nanometer-scale cylinders has been to start with ring-shaped molecules that stack on top of each other. **Huang *et al.*** (p. 1521; see the Perspective by **Zhang and Aida**) took this approach a step further by giving the rings a flexible diameter. Specifically, rings were prepared consisting of six v-shaped building blocks with hydrophobic sides that could slide back and forth along one another and thereby expand or contract the pore at the center. The rings spontaneously stacked to form tubes in dilute aqueous solution, and heating induced contraction of the whole tube in a process that was readily reversible on cooling.

## A Pair of Planets Around a Pair of Stars

Most of the planets we know about orbit a single star; however, most of the stars in our galaxy are not single. Based on data from the Kepler space telescope, **Orosz *et al.*** (p. 1511, published

online 28 August) report the detection of a pair of planets orbiting a pair of stars. These two planets are the smallest of the known transiting circumbinary planets and have the shortest and longest orbital periods. The outer planet resides in the habitable zone—the "goldilocks" region where the temperatures could allow liquid water to exist. This discovery establishes that, despite the chaotic environment around a close binary star, a system of planets can form and persist.

*Continued on page 1432*



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## This Week in *Science*

Continued from page 1431

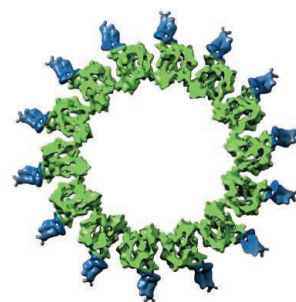
### Identifying BAP1 Targets

Inactivating mutations in the deubiquitinating enzyme BAP1 have been associated with cancer.

**Dey et al.** (p. 1541, published online 9 August; see the Perspective by **White and Harper**) reveal molecular targets of the enzyme and show evidence for a role in leukemia. Mice specifically lacking the target of BAP1, HCF-1, in the bone marrow developed myeloid leukemia. BAP1 appears to be part of a complex that regulates modification of histones and gene expression important for normal hematopoiesis and tumor suppression.

### Motoring Along

Dyneins are large and complex molecular motors that transport cargo along cellular microtubules and power the movement of cilia. An enigma is how microtubule binding and nucleotide hydrolysis are coordinated between sites separated by 25 nm. **Redwine et al.** (p. 1532) report an electron microscopy structure of the dynein microtubule-binding domain bound to microtubules in a high-affinity state and combined this with molecular dynamics and existing x-ray structures to provide a model for how dynein couples its affinity for microtubules with the nucleotide-bound state of the motor domain.



### Fixing on a Marine Partnership

Nitrogen fixation by microorganisms determines the productivity of the biosphere. Although plants photosynthesize by virtue of the ancient incorporation of cyanobacteria to form chloroplasts, no equivalent endosymbiotic event has occurred for nitrogen fixation. Nevertheless, in terrestrial environments, nitrogen-fixing symbioses between bacteria and plants, for example, are common. **Thompson et al.** (p. 1546) noticed that the ubiquitous marine cyanobacterium UCYN-A has an unusually streamlined genome lacking components of the photosynthetic machinery and central carbon metabolism—all suggestive of being an obligate symbiont. By using gentle filtration methods for raw seawater, a tiny eukaryote partner for UCYN-A of less than 3-μm in diameter was discovered. The bacterium sits on the cell wall of this calcifying picoeukaryote, donating fixed nitrogen and receiving fixed carbon in return.

### Removing Fear Memories

Disruption of reconsolidation of an activated fear memory prevents subsequent fear expression. After a memory reminder, extinction training can disrupt fear memory. In rodents, this process is dependent on a brain area called the amygdala. **Agren et al.** (p. 1550) used functional magnetic resonance imaging and a fear conditioning paradigm to show in humans that, after an associative fear memory was formed, reactivation and reconsolidation left a signature in the basolateral amygdala. This memory trace predicted later fear expression, which was linked to activity in areas forming the fear circuit of the brain. Extinction alone did not change this signal. However, extinction in the reconsolidation window blocked fear expression by erasing the fear memory trace in the amygdala and weakened the connection in the wider fear circuit of the brain.

### Recognizing Escaped Commensals

In order to coexist peacefully, the billions of bacteria in our gut and our immune system have reached a détente. An intestinal mucosal firewall exists, so bacteria remain localized to the gut, where the immune system is tightly regulated so that these bacteria are tolerated. Enteric infections, however, lead to a breach in this mucosal firewall, resulting in exposure of the peripheral immune system to the intestinal bacterial contents. What is the result? Using oral *Toxoplasma gondii* infection in mice, **Hand et al.** (p. 1553, published online 23 August) show that, besides the *T. gondii*-specific T cell response, a commensal bacteria-specific T cell response is elicited. The CD4<sup>+</sup> T cell-specific response was tracked to a commensal-derived flagellin, and these T cells expanded after *T. gondii* infection and formed long-lived memory cells able to respond to subsequent challenges. Thus, enteric infections can lead to the formation of commensal bacteria-specific, long-lived memory T cells that reside throughout the body—which may play a role in intestinal pathologies such as inflammatory bowel disease.

CREDIT: REDWINE ET AL.





Ezekiel Emanuel is the Vice Provost for Global Initiatives and chair of the Department of Medical Ethics and Health Policy at the University of Pennsylvania, Philadelphia, PA. E-mail: [vp-global@upenn.edu](mailto:vp-global@upenn.edu).

## Prevention and Cost Control

PREVENTION IS THE KEY TO COST CONTROL AND IMPROVING THE QUALITY OF HEALTH CARE IN MANY nations. Most people think of prevention as vaccines and screening tests. But it is tertiary prevention—keeping people with established diseases from becoming worse—that holds the greatest promise for strengthening the health care system. Why? Health care costs are unevenly distributed across populations. In the United States, 50% of the population uses hardly any health care, whereas 10% consumes nearly two-thirds of all health care spending. The latter are patients with one or more chronic conditions, such as congestive heart failure, diabetes, or cancer. To control costs, we must prevent the conditions of this 10% of patients from worsening.

Primary preventative strategies (treating healthy people to avoid disease), such as vaccination, and secondary strategies (diagnosing and treating people who are at risk of developing disease) remain critical interventions. Tertiary prevention improves the care of patients with serious and often multiple chronic illnesses, and it requires extending responsibility for their health beyond the hospital and physician's office. This approach begins with interventions that transform medical care: entrusting care to multidisciplinary teams that share a common electronic health record with a single care plan; giving the patient access to a health care provider who has the patient's clinical notes, diagnoses, medication list, and care plan; and establishing specialized clinics for recurrent problems. It also requires careful monitoring at home of early physiologic indicators; frequent interactions (in person, by phone, or by e-mail) to enhance patient engagement with their own health through activities such as diet compliance; home services, including house calls for emergencies; education and support for the patient's caregivers and family members; and even transportation services to ferry patients to and from medical appointments.

This type of intensive outpatient care for the chronically ill can achieve remarkable results. For instance, CareMore, a private health plan for seniors, has documented a 56% reduction in hospitalization of patients with congestive heart failure and a 60% reduction in amputations for diabetics.\* Overall, CareMore's Medicare beneficiaries have a readmission rate of 10% as compared to approximately 20% for all Medicare patients (Medicare is the U.S. federal health insurance program for seniors).† Hospital lengths of stay are 38% shorter than the national average. The primary care team and specialists work together closely, avoiding many superfluous tests and treatments. This approach has dramatically improved the quality of care, with cost savings focused in three areas: reductions in emergency room use, hospitalization and readmission rates, and use of specialists. Overall, groups such as CareMore reduce costs by 15 to 20%.‡

The expansion of tertiary prevention presents important challenges. How can proven models be successfully introduced into new settings? Can Medicare, Medicaid, and private payers transform their payment systems to incentivize the appropriate types of services? Groups that have successfully implemented tertiary prevention usually receive global capitation payments that allow them to redirect resources and reward physicians and other providers for improving the health of their patients, rather than rewarding them for treating acute exacerbations. Somehow, health care systems must move away from a fee-for-service payment system that rewards performing more interventions and penalizes a tertiary prevention approach. Any quality health care system must control costs. An effective implementation of tertiary preventative measures is an important step in this direction, while simultaneously improving health.

— Ezekiel Emanuel

10.1126/science.1229493

\*CareMore, *CareMore: A Model for Caring for Those at Greatest Risk* (January 2012); [www.ahip.org/CareMoreSlides.aspx](http://www.ahip.org/CareMoreSlides.aspx).

†R. White, *New Health Care Models Offer Alternative to Fee-for-Service Care* (University of Southern California Reporting on Health, 26 July 2012); [www.reportingonhealth.org/2012/07/26/acos-offer-alternative-fee-service-care](http://www.reportingonhealth.org/2012/07/26/acos-offer-alternative-fee-service-care).

‡A. Milstein, E. Gilbertson, *Health Affairs* 28, 1317 (2009); <http://content.healthaffairs.org/content/28/5/1317.full?sid=fc78ace7-d812-404d-9557-cefdcda8f793>.



EDITED BY KRISTEN MUELLER AND MARIA CRUZ



## DEVELOPMENT

### Surprising Origins

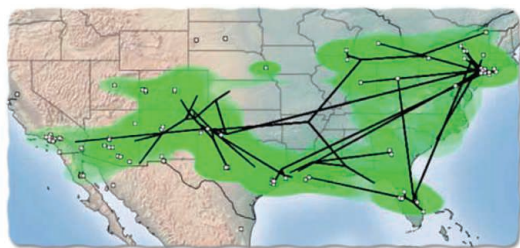
Similarities exist between the inner ear hair cells of humans and the lateral line hair cells of fish. Both respond to mechanical movements, from sound waves or water, respectively, and both arise from placodes of head ectoderm during early development. Humans, however, do not have a lateral line sensory organ. Many amniotes, including birds and some reptiles, have a paratympanic organ (PTO) near the middle ear that similarly contains hair cells and responds to mechanical distortion, suggestive of an evolutionary linkage between the lateral line and the PTO. O'Neill *et al.* now show that these ideas are close to the mark, but not right on. Studying the PTO in chickens, the authors show that the PTO derives from an ectodermal placode close by, but distinct from, the placodes that generate geniculate neurons and the lateral line. Although the PTO and geniculate placodes are so close as to almost blend together, they are nonetheless distinguished by their patterns of transcription factor expression and the developmental progressions that follow. The authors suggest that the PTO placode represents its own developmental module, subject to evolutionary trajectories independent of those of the lateral line. — PJH

*Nat. Commun.* **3**, 1041 (2012).

## EPIDEMIOLOGY

### The Right Time and Place

Spatial analysis, such as that used in epidemiology, is vulnerable to many errors, including that caused by spatial dependence or autocorrelation. For transmission of many infections, individuals need to be neighboring, and because the environment and behavior of nearby surroundings tend to be more similar than distant ones, neighbors will tend to be statistically dependent. This phenomenon causes complications for accurate epidemiological modelling. However,



pathogens can also be mapped in time, as well as place, and evolutionary biologists have developed phylogeographic methods to aid this sort of historical sleuthing. Given current concerns about the unexpected emergence of pathogens such as West Nile virus (WNV) in the United States, Pybus *et al.* have merged these concepts to develop

an alternative, less error-prone approach. Taking data for WNV, they show how the diffusion coefficient and variation in the spatial spread of a pathogen can be estimated from genome data alone. This approach revealed that instead of a steady front of east-to-west dissemination of WNV, it progressed in fact by rare long-range movements, probably triggered by bird migration or anthropogenic transport of mosquitoes. These rare events leave a detectable phylogenetic footprint. Ignorance of this hitherto hidden heterogeneity has led in turn to considerable overestimation of the pathogen's basic reproductive number,  $R_0$ , a key parameter for estimating the epidemic potential of a pathogen. — CA

*Proc. Natl. Acad. Sci. U.S.A.* **109**, 15066 (2012).

## BIOMEDICINE

### The Good and the Bad in ALS

Amotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron death. Development of effective therapies will require an understanding of the molecular and cellular mechanisms that go awry in the disease—insights that often come from genetic approaches. Mutational analyses of rare hereditary forms of ALS have already implicated >12 culprit genes. Although several of these genes converge on common pathways, the overall view of pathogenesis remains incomplete.

Recent studies have uncovered two new genetic mutations that have an impact on ALS; interestingly, in one case the mutations appear to have a salutary effect on the course of the disease. Through exome sequencing of two large ALS families, Wu *et al.* discovered disease-associated mutations in the *PFN1* gene, which encodes the actin-binding protein profilin-1. In cultured cells, mutant profilin-1 formed insoluble aggregates and inhibited axonal outgrowth. Starting with a zebrafish model of ALS, Van Hoecke *et al.* discovered a disease-modifying gene called *EPHA4*, which encodes a receptor tyrosine kinase that interacts with ephrins, proteins involved in axonal repulsion. Inhibition of EphA4 signaling had beneficial effects in fish and rodent models of ALS. Importantly, two ALS patients who carried inactivating mutations in *EPHA4* showed uncharacteristically long survival. — PAK

*Nature* **488**, 499 (2012); *Nat. Med.* **18**, 1418 (2012).

## CELL SIGNALING

### Flip-Flop Messenger

CD38 is a transmembrane protein present on lymphocytes that appears to function in signal transduction. It has multiple enzymatic activities, two of which cause the generation of molecules that function to regulate the release of calcium from intracellular stores [cyclic ADP-ribose and nicotinic acid adenine dinucleotide



phosphate (NAADP)]. Enigmatically however, the catalytic domain of CD38 has been localized to the outside of the cell. Zhao *et al.* used antibodies specific to the N- and C-termini of CD38 to look more closely at its orientation. They found that in multiple cell lines and in primary human peripheral blood mononuclear cells, a few percent of the cells expressed CD38 with the catalytic C-terminal portion of the protein on the inside, with some cells expressing the protein in both orientations. The authors propose that regulated expression of CD38 in its different orientations might provide a mechanism for control of its effects on intracellular signaling. — LBR

*Sci. Signal.* **5**, ra67 (2012).

#### CHEMISTRY

### Fast Protein-Binding Nanoparticles

One possible use of synthetic polymeric nanoparticles is to target and bind protein *in vivo*. As such, these nanoparticles have potential for medical applications, but often their performance is lower *in vivo* than *in vitro* because of slow binding kinetics and nonspecific protein binding. Hoshino *et al.* borrowed the concept of "induced fit" for enzymatic binding to substrates to improve the binding kinetics of a polymeric nanoparticle. They used a poly-*N*-isopropylacrylamide (PNIPam) backbone, which has a temperature-driven phase transition from a flexible random coil to a rigid conformation. The protein target, concanavalin A, binds the sugar mannose. To prepare synthetic polymeric nanoparticles that recognized the target protein through multipoint interactions, the authors synthesized PINPam nanoparticles copolymerized with *p*-acrylamidophenyl- $\alpha$ -mannopyranoside. Nanoparticles in the flexible conformation have faster binding kinetics than those in the rigid conformation, but the fastest kinetics was observed at the transition temperature between the swollen and collapsed phases. — PDS

*J. Am. Chem. Soc.* **10.1021/ja306053s** (2012).

#### OCEAN SCIENCE

### Heavyweight Measurements

One of the potentially most serious consequences of global warming is the rise of sea level that will occur as the polar ice sheets shrink. Part of that sea-level rise will be due to ocean warming, because warmer water occupies a larger volume than an equivalent amount of colder water; the other part will be due to more water in the sea;

i.e., to a larger mass of water. Good measurements of ocean temperatures are available, but how does one go about measuring the mass of the ocean? There may be an easy way, according to Hughes and colleagues from the UK National Oceanography Centre and the School of Civil Engineering and Geosciences of Newcastle University.

It turns out that there are places on the ocean floor where the pressure of the overlying water does not change much in response to the wide array of causes (such as ocean dynamics, tidal forcing, and changes in atmospheric pressure, among others) that can make it vary independently of ocean volume changes. In such a spot, one could theoretically install a single ocean-bottom pressure (OBP) sensor and measure how whole-ocean mass was changing. The authors used models to identify a suitable spot and OBP measurements from the Pacific Ocean to illustrate the technique's potential. If their idea is correct, and if OBP sensors with low enough measurement drift can be developed, there may be a sweet spot for monitoring ocean mass changes. — HJS

*Geophys. Res. Lett.* **39**, L17602 (2012).

#### ENVIRONMENTAL SCIENCE

### Dissolving CO<sub>2</sub> in Brine

Carbon dioxide capture and long-term storage are seen as one way to mitigate and defer global warming. One idea for capturing and storing CO<sub>2</sub> is injecting it into the highly saline groundwaters that are common on many continents. These brines are often relics of earlier hydrologic systems and have persisted for millions to tens of millions of years (or longer) because their high salinity makes them denser than shallow freshwater systems. Using thermodynamic modelling of the system CO<sub>2</sub>-H<sub>2</sub>O-NaCl-CaCO<sub>3</sub>, Steele-MacInnis *et al.* explore what will happen to CO<sub>2</sub> when injected as a supercritical fluid into deep saline formations, with a goal of estimating the available storage volume. Initially, injected CO<sub>2</sub> will displace the brine, but over time, it will dissolve in the brine. Their thermodynamic analysis shows that this dissolution will yield the most favorable storage conditions and will markedly reduce storage volume requirements. It will also increase the density of the brine, stabilizing it further. — BH

*Environ. Sci. Technol.* **10.1021/es301598t** (2012).



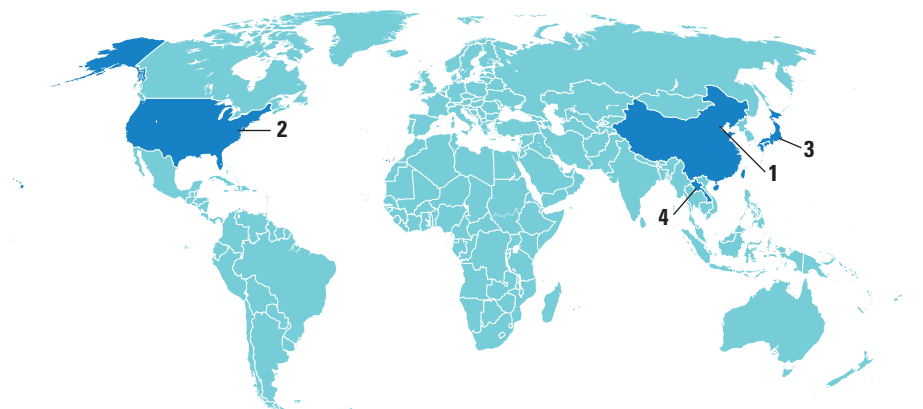
# 22 minutes and 58 seconds of video on accelerated mass loss from Antarctica's ice shelves.

One more data point on why you should spend more time at [membercentral.aaas.org](http://membercentral.aaas.org). There you can enjoy evidence-based videos, webinars, downloads, blogs, and discounts geared for people who live for all things empirical.



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## AROUND THE WORLD



## Beijing 1

**Censured Chinese Official's Promotion Ignites Anger**

An official who was reprimanded after a 2008 scandal involving tainted baby formula has been promoted to deputy director of China's State Food and Drug Administration, sparking calls for transparency. Sun Xianze formerly oversaw food safety for the agency. He was issued an official demerit in 2009, after six infants died and nearly 300,000 fell



sick from ingesting milk powder laced with melamine, an industrial chemical that causes kidney damage. Sun was one of dozens of officials censured in the wake of the tragedy, which also brought a government pledge to improve food safety (*Science*, 28 November 2008, p. 1310).

Under the terms of Sun's demerit, he was not allowed to advance in the agency for 12 months—a term he has since served. Still, his promotion brought public outrage. Li Fangping, a lawyer who defended victims in the scandal, called for greater openness. "There should be public release of his résumé and wrongdoings before the promotion," he told China's *Global Times*.

## Washington, D.C. 2

**A Forbidding Budget Outlook**

A spending freeze for now—but deep cuts may come later. That's the message sent to U.S. government science programs by two recent federal budget developments. This week, Congress was expected to finalize a temporary spending bill that will fund government operations through 27 March 2013. The deal avoids a government shutdown and gives lawmakers more time to resolve spending disagreements over the 2013 fiscal year, which begins on 1 October. This stop-gap measure freezes most agency budgets at current levels (providing a tiny 0.612% increase), but gives the National Oceanic and Atmospheric Administration some extra cash to prevent further delays in two Earth-observing programs, the Geostationary Operational Environmental Satellite-R initiative and the Joint Polar Satellite System. But those and other science programs face major spending cuts if by the end of the year Congress can't reach a long-term deal to reduce U.S. deficits, the White House warned on 14 September. Spending on civilian research would shrink by 8.2%, and defense research by 9.4%, under an existing law that would impose across-the-board cuts on 2 January 2013 unless lawmakers act. Science advocates are imploring lawmakers to prevent the automatic "sequester," arguing it would devastate research. [http://scim.ag/budget\\_woes](http://scim.ag/budget_woes)

## Tokyo 3

**Japan to Phase Out Nuclear Power, End Monju Project**

Japan is embarking on a plan to end the use of nuclear power by sometime in the 2030s and convert the Monju experimental fast breeder reactor into a nuclear waste

transmutation facility with a limited but unspecified lifetime. The policy rethink was triggered by the March 2011 disaster at the Fukushima Daiichi Nuclear Power Plant and reverses a 2010 plan that called for boosting reliance on nuclear power from about 30% to 45% of generating capacity by mid-century. Existing reactors will be shut down after 40 years of operation. The new energy policy, endorsed by a Cabinet panel last week, shifts the focus to conservation and renewable energy sources.

What will happen to nuclear power-related research "is yet to be discussed," says Satoru Tanaka, a University of Tokyo nuclear engineer and former president of the Atomic Energy Society of Japan. It is also possible that the political party that wins the national elections, likely to be held before the end of this year, could revisit the decision to phase out nuclear power. [http://scim.ag/\\_reactor](http://scim.ag/_reactor)

## Laos 4

**Child Mortality Falls, but Not Fast Enough**

The Lao People's Democratic Republic cut its child mortality rate by 72% between 1990 and 2011. That's one of the most dramatic reductions detailed in a report released on 13 September by UNICEF, the World Health Organization, the World Bank, and the U.N. Population Division. Global child mortality has fallen by 41% since



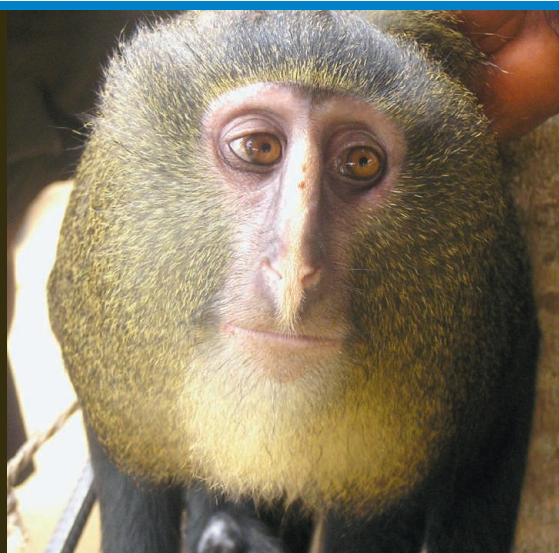
1990, according to the new statistics—good news, but still not good enough to meet the Millennium Development Goal 4, which aims for a two-thirds reduction in child mortality between 1990 and 2015.

Sub-Saharan Africa and Southern Asia



## Pet Monkey Turns Out to Be New Species

Conservation biologist John Hart didn't set out to bag a monkey unknown to science. He wanted to survey bonobos in the unexplored lowland rainforest of the Democratic Republic of the Congo's Lomani basin. But a colleague's photograph of an unusual monkey on a bushmeat hunter's canoe changed his mind. Learning that a schoolmaster's daughter living on the edge of the forest had adopted one of the young monkeys as a pet, Hart—with the Lukuru Wildlife Research Foundation in Kinshasa—and colleagues tracked its growth for the next 19 months. They also observed the primate in the wild. Unlike many of its cousins, the monkey, locally known as *lesula*, spends most of the time foraging quietly on the ground in small groups. Shortly before dawn, they liven up the forest with "boom" calls, but are otherwise hard to detect. On 12 September in *PLoS ONE*, Hart and his colleagues described *Cercopithecus lomamiensis*—the second African monkey to be "discovered" in 28 years. They are now monitoring the wild monkeys with camera traps and are working toward making the area a national park.



accounted for more than 80% of global deaths among children younger than 5 in 2011. Nearly a quarter of child deaths were in India, in part because of its large population. The leading cause of death was pneumonia, responsible for 18% of worldwide deaths in children younger than 5, followed by preterm birth complications, which accounted for 14%.

Besides scaling up basic health services, educating girls and women has significant impact on child survival, the report says.

## NEWSMAKERS

### NIH Picks Translational Center Chief



The National Institutes of Health has chosen developmental neuro-geneticist **Christopher Austin** to direct its 9-month-old National Center for Advancing Translational Sciences (NCATS). He starts on

23 September and will head a \$575 million agency aimed at addressing bottlenecks in the drug development process.

Austin, who turns 52 next week, has spent most of the past decade at the National Human Genome Research Institute, where he launched a set of small-molecule screening centers and headed programs such as drug development for rare and neglected diseases. Before that, he worked on genome-based drug discovery at Merck for 7 years.

Austin described his promotion as the

"culmination" of career-long efforts to bridge basic research and the clinic. "This is a really hard, ambitious, but deeply important mission we're all on," he told NCATS's advisory council last week.

Steven Paul, a former Eli Lilly research chief now at Weill Cornell Medical College in New York City who served on the search committee, says: "We came up with a terrific person. ... Chris has credibility with both academic investigators and the private sector." <http://scim.ag/NCATSchief>

### Three Q's

The National Academy of Engineering (NAE) has nominated mechanical engineer **C. Daniel Mote Jr.**, former president of the University of Maryland, College Park, from 1998 to 2010, as its next president. If elected by the academy's membership, Mote will succeed current president Charles Vest when Vest's term ends on 30 June 2013.



**Q: What challenges does the academic engineering community—and the U.S. engineering enterprise—face today?**

Many of the challenges they face are related to funding, but also the national commitment to basic research and the competitiveness of U.S. research globally. Since 1990, the world has been moving into a globalization mode. It is no longer about controlling information and products. Now, the game is to engage globally to develop your products.

**Q: What will your priorities be as president of the academy?**

We need to capitalize on NAE programs that create a flexible and agile engineering talent base. We also need to update continuing education efforts. This responsibility has to fall on universities and colleges in the coming years. They have traditionally seen this as an auxiliary responsibility but that needs to change.

**Q: Will international collaborations help or hurt efforts to foster innovation in the United States?**

There is a tendency in this country to think that collaboration means the U.S. gives away knowledge. With collaborations, everybody gets something. It's hard to sell products to different countries if you don't understand what they want and why. If people spend time in different countries, you understand this picture a lot better.

<http://scim.ag/NAEhead>

## NOTED

**>Amy Bishop**, a former assistant professor of biology at the University of Alabama, Huntsville, who shot and killed three fellow faculty members and injured three more in 2010 after being denied tenure, **pleaded guilty last week to capital murder charges**. Under the terms of the plea, she'll avoid the possibility of the death penalty and will instead be sentenced to life in prison. [http://scim.ag/\\_Bishop](http://scim.ag/_Bishop)

## FINDINGS

## Hail Mary: Reptile Style

Virgin births aren't unheard-of among animals that reproduce sexually. But they are rare and were thought to occur only in captivity. So researchers studying the breeding habits of wild cottonmouth and copperhead snakes—types of North American pit viper—were surprised to discover that 2.5% and 5%, respectively, of the litters produced by wild female snakes did not involve males. “To my knowledge this is the first time it has been described from a wild-caught litter,” says geneticist Kevin Feldheim of The Field



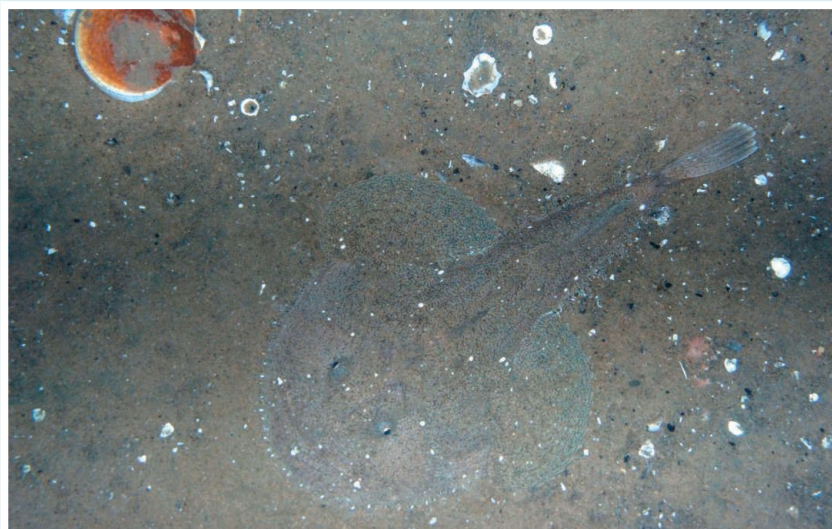
Museum in Chicago who was not involved with the work. While evaluating data from two long-term studies of breeding in snakes,

Warren Booth of the University of Tulsa in Oklahoma and colleagues studied 22 copperhead litters collected in Connecticut, and 37 cottonmouth litters collected in Georgia. They noticed that two litters—one from each species—were very small and had developmental failures that might be signs of virgin births, or facultative parthenogenesis. Genetic testing proved that both litters had no input from males, the team reported online on 12 September in *Biology Letters*. The next step, Feldheim says, is to see whether those all-male litters are fertile.

## Random Sample

## Citizen Science on the Sea Floor

Calling all fish finders and scallop spotters: Seafloor Explorer needs your help! Launched on 13 September, this interactive Web site asks ordinary people to help spot interesting critters and objects in photographs of the ocean floor. Researchers hope these citizen scientists can help them analyze the deluge of images (some 3 million per day) captured by an underwater vehicle called the HABitat mapping CAMera System (HabCam). Towed over the sea floor along the U.S. East Coast, HabCam has produced more than 500 million pictures that need analysis. For now, just 100,000 of them have been uploaded to the Seafloor Explorer site, but scientists plan to add more soon. “The information that we’re gathering, at least initially, is to help support our ability to collect information on the interaction between different organisms, like sea stars and their predators,” says biologist Scott Gallagher of the Woods Hole Oceanographic Institution in Massachusetts. Gallagher also hopes that once enough pictures are analyzed, he can use the database to develop an automated classification system for HabCam’s images. Seafloor Explorer is the latest crowdsourcing project from Citizen Science Alliance (CSA), the team behind Galaxy Zoo and other citizen science initiatives available on Zooniverse.org. “The techniques have been refined over a number of projects now, and seem to produce excellent science,” says Arfon Smith, CSA director. Zooniverse projects have resulted in at least 40 peer-reviewed papers, and Smith is confident that the data from Seafloor Explorer will bump that number even higher. Check out Seafloor Explorer at <http://www.seafoorexplorer.org/>.



## BY THE NUMBERS

**56.7°C** Hottest official temperature on Earth, recorded in Death Valley in 1913. In a 13 September announcement, the World Meteorological Organization (WMO) stripped Libya of its 90-year claim to the title after a WMO investigation concluded that the country’s chart-topping 58°C temperature had been recorded incorrectly.

**1.2 million square kilometers** Projected increase in global urban land area by 2030. This would triple the global coverage recorded in 2000, according to a study published online on 17 September in the *Proceedings of the National Academy of Sciences*.

## Science LIVE

Join us on Thursday, 27 September, at 3 p.m. EDT for a live chat on **the scientific job market**. <http://scim.ag/science-live>



INFECTIOUS DISEASES

# New XMRV Studies Bring Closure—and Fresh Dispute

Most of the scientific community long ago pronounced dead the theory that a newly discovered gammaretrovirus, dubbed XMRV, was linked to chronic fatigue syndrome (CFS). The idea, launched in a *Science* paper in 2009, quickly garnered headlines because it might explain the baffling disease and suggest ways to treat and prevent it. But since then, study after study has demolished the claim, and last year, *Science* retracted the paper (23 December 2011, p. 1636).

But the results of the biggest study of all had yet to come out. Funded by the U.S. National Institutes of Health (NIH) and led by Ian Lipkin of Columbia University, the \$1 million multicenter project finally published its results on Tuesday in *mBio*—and not surprisingly, it concludes that the XMRV theory is really, really dead. What is surprising, scientists say, is that Judy Mikovits, the main author of the 2009 paper and the staunchest defender of a role for XMRV—or something closely related—is won over. Mikovits, who participated in Lipkin's study, concedes it is "the definitive answer. ... There is no evidence that XMRV is a human pathogen."

Meanwhile, another group has also wrapped up some unfinished business. XMRV was first reported in *PLoS Pathogens* in 2006 by Robert Silverman of the Cleveland Clinic in Ohio, along with colleagues at the University of California, San Francisco (UCSF); at the time, they linked the virus to prostate cancer. A new paper by many of the same authors, published in *PLoS ONE* this week, soundly refutes that link as well and describes their meticulous detective work to explain how the spurious findings arose.

But these authors have sparked a new controversy by saying that XMRV remains a potential pathogen and refusing to retract

their 2006 paper. Indeed, two high-impact journals gave the new paper a thumbs-up but refused to publish it unless the authors retracted their original work, says UCSF's Charles Chiu, who didn't participate in the 2006 study but whose lab did most of the analyses for the new paper.

Lipkin's study is one of two similar projects NIH funded after Mikovits published her 2009 paper. One, by the Blood XMRV Scientific Research Working Group, set out to discover if labs could reliably detect XMRV infection in people and whether the U.S. blood supply was in danger. The Lipkin study, which used 10 times the number of samples, was designed to get a definitive answer on the link to CFS.

To most scientists, the blood study, in which Mikovits also participated, definitely ruled out XMRV as a pathogen—even more so because a 2011 paper by John Coffin and Vinay Pathak of the National Cancer Institute (NCI) had shown that the virus was a hybrid of two mouse viruses, accidentally created in the lab in the 1990s (*Science*, 23 September 2011, p. 1694). As a result, NIH received an "enormous amount of criticism" for continuing his study, Lipkin says. But Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, says it was worth going the extra mile to disprove it, especially because

**What went wrong?** Robert Silverman had to figure out how XMRV contaminated his samples.

CFS patients had become so emotionally invested in the XMRV theory. "Now, it's clear to everybody that it is really over," Fauci says.

Three groups took part in Lipkin's study: Mikovits, formerly at the Whittemore Peterson Institute (WPI) in Reno, Nevada, and her collaborators Francis Ruscetti at NCI and Maureen Hanson at Cornell University; a team led by Shyh-Ching Lo at the Food and Drug Administration, which in 2010 linked CFS to a related group of viruses called MLVs (*Science*, 27 August 2010, p. 1000); and a group at the Centers for Disease Control and Prevention that had failed to find any new virus in CFS patients. The samples were blinded, and each team chose their own methods to analyze them so no one could complain that the right procedures weren't used, Lipkin says.

This time, none of the groups found any evidence of XMRV or MLVs in 147 patients or 146 controls. Mikovits and Ruscetti did find that about 6% of patients and controls had antibodies to XMRV, a result they chalk up to aspecific binding effects rather than XMRV infection. No previous study had tried to replicate her findings using her exact methods, Mikovits says. "I'm forever grateful to Ian Lipkin for making it possible to participate," she says. Lipkin says he is "proud" of Mikovits for accepting the outcome.

The controversy around CFS had its origins in the 2006 study by Silverman and the



**Final answer.** Judy Mikovits (left) says she's "forever grateful" to Ian Lipkin (right), who led a big study of the link between XMRV and CFS.

UCSF team that first reported XMRV in prostate cancer patients. As the link to CFS unraveled, Silverman realized that his work might also have problems, he says, and the 2011 paper by Coffin and Pathak convinced him his results were due to contamination. From then on, "I felt like I couldn't rest until

CREDITS: (TOP) COURTESY OF CLEVELAND CLINIC; (BOTTOM LEFT) J. COHEN/SCIENCE; (BOTTOM RIGHT) CENTER FOR INFECTION AND IMMUNITY

I figured out how it happened,” he says.

The researchers took tumor samples from 39 new prostate cancer patients and tested them for XMRV using three different techniques; they also went back to tumor tissue still available from the 2006 study. This time, they found no XMRV in any of the samples. They did find it in archived RNA extracts from the 2006 study, indicating that contamination had happened during sample processing.

Further studies—some using techniques unavailable at the time of the original study—revealed that the virus originated in LNCaP, a cell line infected with XMRV that Silverman’s lab used for other studies. The LNCaP cells, in turn, had become contaminated by 22Rv1, another widely used cell line that also harbors XMRV.

Silverman’s group “deserves a medal,” says Kim McCleary, head of the CFIDS Association of America, a patient advocacy group. In the long history of pathogens falsely blamed for CFS, McCleary says she’s never seen scientists so scrupulously retrace their steps. “These scientists put their egos aside ... to get to the truth,” Pathak adds.

But others are less charitable. In the discussion of the paper, the authors say that XMRV is still a “genuine infectious agent” with “as-yet undefined pathogenic potential”; they point out that the virus is able to infect two primate species and mice and has interesting biological properties that may be useful, for instance, in cancer research. Lipkin says he’s “astonished” by that claim. An accidental lab creation not occurring in nature is not a genuine infectious agent, he says, and he worries that the language may inspire new hope in XMRV believers: “I thought we were really done with this.”

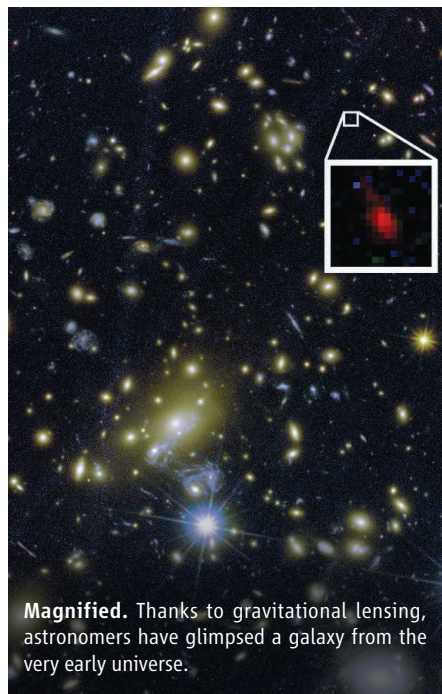
Both Lipkin and Coffin say the 2006 paper should be retracted because its findings were wrong. But Silverman, who retracted his lab’s share in the Mikovits study 2 months ahead of the retraction of the entire paper, says the 2006 study reports the discovery of a new virus—and the rest has been corrected. There have been other cases in which authors corrected, rather than retracted, a spurious finding, he points out. Chiu adds that it would be odd for future papers on XMRV to refer to a retracted paper as a description of the discovery of the virus.

Pathak says that although retraction would be the best option—“just for the sake of setting the record straight”—it may not make much of a difference; any decent scientist interested in the 2006 paper would find the new one as well, he says. Silverman and his colleagues, he says, “have already done the hard part.”

—MARTIN ENSERINK

## ASTRONOMY

## Warped Light Reveals Infant Galaxy On the Brink of the ‘Cosmic Dawn’



Sometimes nature gives you free of charge a discovery you expected to cost billions of dollars. Just ask Wei Zheng and his fellow astronomers, who recently spotted a galaxy dating back to a mere 500 million years after the big bang.

The galaxy, some 13.2 billion light-years from Earth, sets a new record for most distant object sighted by astronomers. Such distant, ancient images are technically beyond the reach of existing telescopes. Imaging the infant universe is a primary goal of the James Webb Space Telescope (JWST), being built at a cost of \$8.7 billion and expected to launch in 2018.

Yet Zheng, a researcher at Johns Hopkins University in Baltimore, Maryland, and colleagues got a sneak preview thanks to gravitational lensing: an effect in which gravity’s ability to bend light turns weighty objects such as galaxy clusters into magnifying glasses for sources behind them. The young galaxy showed up in images taken by the Hubble Space Telescope because the massive gravity of an intervening cluster magnified it more than 15 times. “We got this image without additional funding from Congress,” Zheng says in a joking reference to JWST, whose ballooning cost has forced NASA to ask appropriators for extra cash

(*Science*, 19 November 2010, p. 1028).

From studying the image, the researchers estimate that the galaxy is less than 200 million years old and formed more than 300 million years after the big bang. Its estimated 100 million solar masses’ worth of stars makes it just 1% as massive as the Milky Way.

In the timeline of cosmic evolution, the galaxy represents an era that is still filled with mystery. The universe was a soup of hot plasma for a few hundred thousand years after the big bang. Then the electrons and protons in the soup combined to form hydrogen. The first stars and galaxies are believed to have been born some 300 million years after the big bang. Over the next 700 million years or so, something reionized the universe, breaking its hydrogen back into electrons and protons.

Studies of the cosmic microwave background have broadly confirmed this timeline. But key early details are missing, including what led to the reionization. Many astrophysicists have suggested that ultraviolet (UV) radiation from early galaxies may have played an important role.

Zheng and his colleagues say that their discovery of the faint galaxy supports that idea. Because the magnifying glass that helped bring their galaxy into view covers only a small volume of the sky, Zheng says, it is possible that many other such galaxies were around at the time.

“Theoretical models of reionization associate most of UV production with galaxies of this or somewhat lower masses at exactly the same cosmic time,” says Avi Loeb, an astrophysicist at Harvard University. “However, with only one galaxy at hand, it is difficult to draw robust statistical inferences.” Loeb says ongoing lensing surveys or future telescopes—such as JWST—will help astronomers determine whether such galaxies were indeed the primary sources of ionizing radiation “at cosmic dawn.”

Rogier Windhorst, an astronomer at Arizona State University, Tempe, and a member of JWST’s science team, calls the discovery impressive but adds that “we definitely need JWST to find the main population of these objects,” including more distant ones, and determine their physical properties.

—YUDHIJIT BHATTACHARJEE



## ARCHAEOLOGY

# Did Neandertals Truly Bury Their Dead?

**LA FERRASSIE, FRANCE**—The French Dordogne is known for its hearty wine, rich foie gras—and spectacular prehistoric finds. This hamlet is home to one of the most famous: During excavations here beginning more than 100 years ago, French archaeologists discovered the skeletons of seven Neandertals, including four children and infants, and the most complete adult Neandertal skull ever found. They concluded that all were deliberately buried, making this site pivotal to contentions that Neandertals had symbolic capacities.

Until now, that is. New excavations at La Ferrassie, co-directed by archaeologists Alain Turq of the National Museum of Prehistory in nearby Les Eyzies-de-Tayac and Harold Dibble of the University of Pennsylvania, are in part designed to reexamine this question, which many researchers had long thought was itself dead and buried. “People are starting to talk about Neandertal burials again,” Dibble says. “It’s getting heated.”

The stakes are high: Most archaeologists still think that Neandertals engaged in mortuary rituals like modern humans do, which means that they shared with our species a richly symbolic activity. “This is a critical issue,” says archaeologist Paul Pettitt of the University of Sheffield in the United Kingdom, who is not a member of the team. “Burial is thought to be a symbolic act in itself and thus is highly pertinent to our evaluation of Neandertals’ symbolic abilities” and cognitive capabilities (*Science*, 10 August, p. 642).

To find out more about how the La Ferrassie skeletons were buried, and whether they were deliberately placed or washed in from a higher point, the team has opened new excavations immediately adjacent to where two adult Neandertals were found. They’re conducting microscopic studies of the sediments and comparing them to sediments clinging to a foot bone uncovered in the original excavations. Of course, new fossils may also help, and last month the team dug up a human heel bone in the new excavation area, though more analysis is required to confirm it as Neandertal.

The roughly 30 team members reflect the field’s broader debate, for despite collegial working relationships, they are deeply divided on the burial question. Turq and many other French members of the team see no reason to question the dominant paradigm that Neandertals, like many prehis-

toric modern humans, buried their dead. But Dibble and his North American colleagues do question it. “La Ferrassie has always been considered the mother of ritual burial,” Dibble says, “but how much of that is interpretation versus real evidence on the ground?”

The North Americans say their point of view was bolstered by the team’s excavations at the nearby Neandertal site of Roc de Marsal. There, a complete skeleton of a Neandertal child found in 1961 was long considered to be strong evidence for burial. But Dibble and his colleagues, including geoarchaeologist Paul Goldberg of Boston University, applied micromorphology—a relatively new approach that puts entire archaeological sites under the microscope to find clues to how bones and artifacts were deposited—and concluded that Roc de Marsal may not have been a deliberate burial after all (*Science*, 20 November 2009, p. 1056, and 9 December 2011, p. 1388). In a paper published last year in the *Journal of Human Evolution (JHE)*, Goldberg and some other team members argued from a microscopic and macroscopic study of the

protected” by some sort of burial practice that included covering the body with earth. Maureille agrees, adding that parts of the skeleton such as the lower vertebrae would be particularly susceptible to coming apart once the soft body tissues disintegrated if it were not deliberately buried. Maureille adds that the issue of whether the pit was natural or dug by Neandertals is not relevant, because the body could have been deposited deliberately in a natural cavity. Pettitt, in recent publications, has argued that disposing of bodies in natural depressions is a form of “funerary caching,” and that the deliberate digging of graves may have developed later as a way of artificially creating such burial spaces.

A core issue in the debate is the criteria that should be used to define a deliberate burial, and how well they are fulfilled at the approximately 20 Neandertal sites where burial has been claimed. Traditionally, these have included whether a skeleton has been found in a deliberately dug pit or a natural depression; whether the bones are articulated, suggesting that they were protected from scavengers; the position of the



sediments in and around the burial site that the pit in which the child was found was a natural depression, and that its body, which was lying face down, may have slid down into the pit from above.

Turq and team member Bruno Maureille of the University of Bordeaux in Talence, France, were not convinced, however, and declined to sign the *JHE* paper. “We completely agree with the observations, but we disagree on their interpretation,” Turq says. In Turq’s view, a skeleton found intact—as was mostly the case at Roc de Marsal—“automatically indicates the corpse was

body; and the presence or absence of “grave goods,” such as stone tools, that might suggest ritual.

Back in 1989 and 1999, archaeologist Robert Gargett, formerly of the University of New England in Armidale, Australia, contended that none of these criteria were fully met in Neandertal burials. But at the time, most archaeologists rejected Gargett’s arguments. “They were based on nothing, no data,” Maureille says. Gargett, now with the Ronin Institute for Independent Scholarship headquartered in Montclair, New Jersey, praises Dibble and his colleagues for “hav-

ing the audacity” to reopen the question at Roc de Marsal and La Ferrassie.

Within the team today, the clashing views come down to different notions about the default hypothesis: Turq, Maureille, and other like-minded researchers say that for relatively intact Neandertal skeletons, the default hypothesis should be that they were buried deliberately. But other team members start with the opposite view. “The default hypothesis is that it’s not a deliberate burial unless you have positive evidence that it is,” says archaeologist Dennis Sandgathe of Simon Fraser University in Burnaby, Canada, who was the first author of the *JHE* paper.

Dibble thinks the key question is not whether a burial was deliberate, but whether archaeologists confront “a burial or a funeral.” A burial, Dibble says, is simply a “disposal” of a body, while a funeral, com-

plete with ritual activity, is a real “symbolic” act. An additional criterion is whether a “cultural pattern” can be detected, says team member Shannon McPherron, an archaeologist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Prehistoric modern human burials, particularly those more recent than the time of the Neandertals, routinely include beads and red ochre, but “there is no patterning in this [Neandertal] stuff,” McPherron says.

But Pettitt, like many others who are not ready to embrace the doubts of Dibble and



**Was I buried, or not?** Some researchers say that the Roc de Marsal Neandertal child (reconstructed, right) was not buried deliberately.

his colleagues, says that “we archaeologists can set the bar too high.” The only “serious way to deal with this issue,” he says, “is to excavate.” And that is just what the team at La Ferrassie is doing, as it attempts to figure out how seven individuals found here a century ago came to this last resting place.

—MICHAEL BALTER

## LASER FUSION

# Ignition Facility Misses Goal, Ponders New Course

The National Ignition Facility (NIF), a \$3.5 billion laser fusion lab in California, looks certain to miss its deadline at the end of this month for achieving ignition, a self-sustaining fusion reaction that yields more energy than was put in to make it happen. This milestone is considered key for NIF’s twin goals: demonstrating the feasibility of fusion energy, and ensuring the reliability of the U.S. nuclear weapons stockpile. By law, the National Nuclear Security Administration (NNSA), part of the U.S. Department of

Energy, has until 60 days after the deadline to produce a report explaining what barriers to ignition remain, how they can be overcome, and what implications there are for the stockpile.

Managers at Lawrence Livermore National Laboratory, the home of NIF, are playing down the significance of the end of the National Ignition Campaign (NIC), the series of experiments due to run until the end of fiscal year 2012 on 30 September. “The NIC is a milestone, and we’re not going to

achieve that milestone. But we will continue to explore and continue to do ignition science experiments,” says Livermore Director Penrose Albright. Others view the missed deadline differently. “It’s going to be a big deal here,” says a congressional aide who asked to remain anonymous.

Meanwhile, to prepare the report for Congress, dozens of researchers from five NNSA-funded national laboratories and from industry are examining NIC in detail and may recommend a new direction for research at

NIF. “We’re working very hard to describe the state of understanding and the path forward,” says Mary Hockaday, deputy associate director for weapons physics at Los Alamos National Laboratory in New Mexico, who is leading the first draft of the report.

NIF uses an approach called inertial confinement fusion (ICF) in which a huge laser—NIF’s is the most energetic in the world—fires beams from many directions at a tiny capsule containing a mixture of the hydrogen isotopes deuterium and tritium. The powerful laser pulse causes the capsule to implode, crushing the hydrogen fuel to a density 100 times that of lead and heating it to millions of degrees. In theory, the hydrogen nuclei should fuse to



**Dead center.** At the end of a positioner arm, the tiny target sits in the center of NIF’s 10-meter-wide reaction chamber.

CREDITS (TOP TO BOTTOM): LAWRENCE LIVERMORE NATIONAL LABORATORY; ©PLAILLY/ATELIER DAYNES/EURELIOS



produce helium, neutrons, and a lot of energy.

The hard part is getting the capsule to implode smoothly and symmetrically enough to create a central hot spot where fusion will ignite. The burn should then provide its own heat as it propagates outward, consuming most of the fuel. Researchers have been trying to perfect the process since the 1960s, but so far the energy output has fallen far short of the energy of the laser pulse that causes the implosion—1.8 megajoules in the case of NIF's laser.

NIF has been controversial from the start. When first proposed in the early 1990s, it marked a huge leap over existing technology. What made it fly was that Livermore gave it two roles: showing that fusion power plants are possible, and recreating the conditions in a nuclear explosion so weapons scientists can validate their computer simulations and gauge how well sensors and components withstand blasts. (The United States stopped conducting actual nuclear tests in 1992.)

Opponents of NIF, including researchers at other ICF labs, argued that NIF had chosen the wrong laser technology and type of target, and they contested NIF's value for stockpile stewardship. "I've expected this day," says Christopher Paine, head of the nuclear program at the Natural Resources Defense Council, an environmental group in Washington, D.C. "A number of experts have predicted it would be a boondoggle. Solid-state lasers are a dead end."

Serious technical problems during NIF's construction delayed completion by 7 years and more than tripled the cost (*Science*, 17 April 2009, p. 326). In 2005, to keep things on track, the NNSA adopted the NIC: a 2-year road map to ignition. NIC included preparatory experiments at other facilities and relied heavily on computer simulations. Since experiments began at NIF in 2010, NIC has taken up 80% of the facility's laser shots.

Despite widespread praise for the quality of the facility, Mother Nature has refused to play along. Numerous unforeseen physical effects have slowed progress toward ignition (*Science*, 28 October 2011, p. 449). Scientists chart progress with a measure called the experimental ignition threshold factor (ITFX). An ignited plasma would have an ITFX of 1 or greater. When NIC experiments began, the ITFX was 0.001; now it is 0.1 but has stubbornly remained there since last year. Paine is scathing: "Since there is no ignition, let alone gain, even the value of the facility for stockpile stewardship is compromised. They've wasted over \$5 billion."

Weapons scientists and basic researchers are doing valuable work on NIF because,

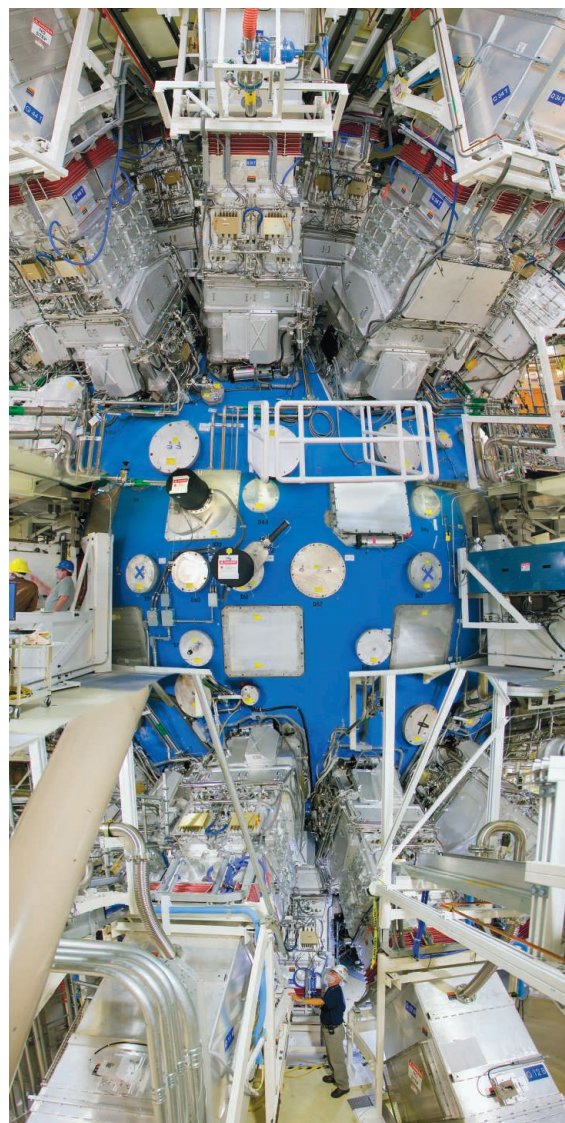
even without ignition, it can produce unprecedented conditions of temperature and pressure, but the congressional aide estimates that if ignition remains elusive NIF will start to outlive its usefulness for stockpile stewardship in a couple of years.

The most recent internal NNSA review of NIF, dated 19 July—aggregating the views of 10 experts from other labs and universities—concluded that the probability of achieving ignition before the end of December is "extremely low." Even the lesser target of observing helium nuclei from fusion reactions heating the surrounding fuel was deemed "challenging." The reviewers highlighted several problems, but their most pressing concern was that Livermore's computer simulations were not accurately predicting what the researchers were seeing. The simulations say that the shots NIF is doing now should be igniting, but the ITFX values show they are far from it. This mismatch means the simulations "are of limited utility in choosing the next set of experiments to perform," the reviewers said.

Christopher Deeney, assistant deputy administrator for stockpile stewardship at NNSA, says managers there decided in the spring that a new approach was needed. A workshop held in May identified 19 areas that require more research. And now the researchers drawing up the report for Congress are also looking at alternative approaches for NIF's future. One is "direct drive," in which the laser beams shine directly onto the capsule. (In NIF's indirect-drive method, the beams illuminate a metal can around the capsule, causing it to emit x-rays that then cause the implosion.) Direct drive is championed by other ICF labs such as the University of Rochester's Laboratory for Laser Energetics and the Naval Research Laboratory. It gets more of the beam energy onto the capsule but requires higher beam quality to ensure symmetry. Deeney says some direct-drive experiments are planned at NIF in 2013.

The specialist groups that have been working on the report to Congress delivered their conclusions to Hockaday and her fellow "ICF execs" from the other national labs on 14 Sep-

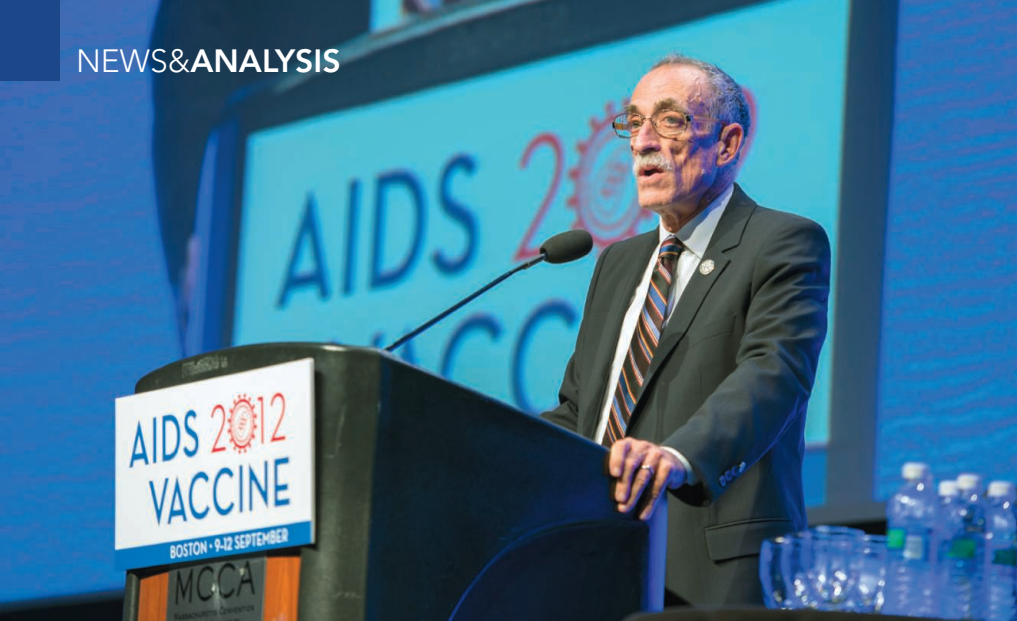
tember, and the execs must deliver a first draft of the report to NNSA by 1 October. What future path NIF will take will have to wait for Energy Secretary Steven Chu to deliver the report to Congress on 30 November. It seems certain, however, that the schedule-driven and simulation-reliant approach of the NIC will give way to a more scientifically methodical one. Deeney says the program will adopt a "discovery science mode, to find out why there is a difference between prediction and experiment."



**All change?** Optical assemblies that guide NIF's laser beams might need costly reconfiguration if the lab shifts to direct drive.

Whatever happens, the pace of progress toward ignition is likely to slow as weapons scientists are clamoring to get more time on the machine. Having made do with 20% so far, they will be getting more than 50% of the shots starting in January 2013.

—DANIEL CLERY



**Change agent.** Erstwhile AIDS activist Bill Snow now heads the Global HIV Vaccine Enterprise.

collaboration” about one of the most exciting leads being pursued, so-called broadly neutralizing antibodies, or bNAbs.

To date, antibodies made by vaccines have had little power against HIV. But for several years, researchers have isolated bNAbs from infected people that, in test tube experiments, work at low concentrations against a wide range of HIV strains. So far, however, no one has figured out how to make a vaccine that triggers bNAbs.

At the meeting, William Schief of the Scripps Research Institute in San Diego, California, showed how his lab has made progress in reverse-engineering a part of HIV that prods the body to make bNAbs. An immunologist who trained as a physicist, Schief started by analyzing a crystal structure that contained a small piece of HIV’s surface protein bound to a bNAb. This HIV peptide, he reasoned, might be the basis of a vaccine if presented to the immune system in the precise shape that it attaches to the bNAb, which he determined using a sophisticated computational program called Rosetta. He then plopped the peptide into a protein scaffold to stop it from wobbling. But when injected into rabbits, it didn’t produce bNAbs.

Rather than scuttle the concept, Schief tested it in a simpler system, respiratory syncytial virus (RSV). At the meeting, he showed that the rabbits injected with the stabilized RSV peptide made bNAbs that worked in test-tube experiments. “It gives us more confidence that it’s possible to make a vaccine this way,” said Schief, who is reassessing why the HIV experiment failed.

The enterprise’s Snow is also pushing the field to resolve long-standing debates. For 3 years, researchers have argued about the “Thai vaccine trial,” which showed marginal efficacy (*Science*, 2 October 2009, p. 26). Presentations at the meeting—and a paper published online on 10 September by *Nature*—described new evidence that backs the contention that the vaccine protected some people because of an antibody it triggered to a region of HIV’s surface protein known as V2. Other developers pay little heed to V2 and shrug at the new data. Much is on the line, as the U.S. military and its partners believe the concept deserves large new trials in both Thailand and South Africa. Snow said it’s high time that V2 idea is either embraced by the field or “cast aside.”

—JON COHEN

## INFECTIOUS DISEASE

# An Enterprising Time for HIV Vaccine Research

**BOSTON**—Teaching the immune system how to outwit a virus that itself survives by outwitting the immune system is a huge scientific hurdle—so much so that several leading AIDS vaccine developers have fled the field. But those who have stuck it out and the younger investigators who have joined them demonstrated at the AIDS Vaccine 2012 conference held here last week that persistence may finally be paying off. Several presentations offered new details about how HIV and the immune system interact, and others proposed novel vaccine design ideas. But the meeting’s most important outcome was less tangible: a new commitment to better coordinate this high-stakes hunt.

That new commitment is largely coming from Bill Snow, recently appointed head of the Global HIV Vaccine Enterprise, which aims to accelerate the search for this preventive tool. But the enterprise, a nonprofit based in New York City launched in 2005 and endorsed by the G8 group of industrialized nations, has never lived up to its promise. Snow, who co-organized the meeting, has bold plans—some already put into action—to stimulate collaboration, make better use of funding, and foster information sharing. “Tonight, quite literally, I’m taking you to task,” said Snow at the meeting’s opening ceremony in a talk that criticized the field for moving too slowly.

Unlike his predecessors, who were all distinguished scientists, Snow, who is HIV-infected, has no formal scientific training. He came to the field as an AIDS activist in 1989,

later helping to found the AIDS Vaccine Advocacy Coalition and sitting on prominent scientific AIDS vaccine committees. Nelson Michael, head of the U.S. Military HIV Research Program, says Snow “brings moral authority” to the task. Carl Dieffenbach, who heads the Division of AIDS at the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, says there’s also an advantage to having a nonscientist in charge. “He rationally can see across the entire discipline, from basic to clinical research, and calls people out when they start talking about things that aren’t going to advance the field,” he says.

The enterprise was initially proposed in a *Science* Policy Forum in 2003 co-authored by Nobelists David Baltimore and Harold Varmus, leading vaccine manufacturers and researchers, and public health leaders (27 June 2003, p. 2036). They argued that the field should divvy up work along the lines of the Human Genome Project, with researchers and funders agreeing on a scientific road map that spelled out priorities. Little of that ambitious vision occurred.

Snow says a key aim is “strategic convening,” which led him to gather Michael, NIAID’s Dieffenbach, and representatives from nine other major funders of HIV vaccine research for 5 hours to discuss priorities, rationales, and budgets. “It was pretty spectacular,” Dieffenbach says, noting that funders typically speak to each other one-on-one. “It’s a different dynamic when you’ve got everyone in the same room.” One immediate outcome, he says, is “a new level of



## ENVIRONMENTAL HEALTH

# Cancers Join List of Illnesses Linked to 9/11 Attacks

Lung cancer, maybe, but will some New Yorkers develop breast cancer and childhood cancer from the toxic dust created by the 9/11 terrorist attacks? That's one question raised by the government's decision last week on the eve of the 11th anniversary of the World Trade Center (WTC) attacks to provide medical care to firefighters and others exposed to the dust who develop any of more than 50 cancers. Although some epidemiologists have criticized the decision, others say it reflects an acceptable approach to evaluating the scientific evidence.

The collapse of the WTC's twin towers created a huge cloud of smoke, fumes, and dust laced with asbestos, metals, and other harmful chemicals. Many workers at the site and survivors developed respiratory problems that researchers have tied to the dust. In 2010, Congress passed a law called the Zadroga Act that created a \$4.3 billion World Trade Center Health Program to care for first responders and people in lower Manhattan on 9/11 lacking insurance who develop certain conditions including lung disease. Those with diseases on the list can also apply for financial compensation.

The Zadroga Act also asked the National Institute for Occupational Safety and Health (NIOSH) to look at cancer. But in July 2011, John Howard, NIOSH's director, found after a review by his staff that there was "insufficient evidence" to support a cancer link in part because of the long lag time for cancer to develop.

Then in September 2011, *The Lancet* published the first epidemiological study tying cancer to the disaster. The NIOSH-sponsored study found 32% more cancers after 7 years among New York City firefighters who worked at the site compared to non-exposed firefighters; the difference was 19% after correcting for the extra surveillance for cancer in the exposed workers. New York lawmakers filed a petition that, under the Zadroga law, required NIOSH to form a scientific panel to rereview the evidence within 180 days.

That panel reported in March that it "believes that exposures resulting from the collapse of the buildings and high-temperature fires are likely to increase the probability of developing some cancers." The panel, made up by law of advocates and a mix of scientists from various fields, based its conclusion largely on evidence that workers and survivors were exposed to known carcinogens and that many had lung or digestive

tract inflammation, which have been linked to cancer. It recommended that 58 cancer types as well as rare and childhood cancers be added to the list of WTC-related conditions. The panel included breast cancer, for example, because the dust contained polychlorinated biphenyls and because women firefighters did shift work, both of which have been linked to this cancer.

Howard followed up with a final rule last week adding the same list of cancers. The agency relied on the advisers' report and its own criteria that a cancer should be listed if it has been tied to a carcinogen in the WTC dust or if epidemiologic evidence links it to 9/11 exposures. Yet the notice acknowledges the lack of hard evidence: Information on individual exposures "is extremely limited" and "its absence impairs definitive scientific analysis of the relationship between exposures arising from the attacks and the occurrence of any specific type of cancer," the rule states.

The scientific committee's chair, epidemiologist Elizabeth Ward of the American Cancer Society, defends her group's report. "It's the best scientific approach one can use when one doesn't have direct data. We're making inferences from other studies and accumulated knowledge to make the best conclusions we could," she says. The same reasoning led to the 1964 U.S. Surgeon General report linking smoking with cancer, she points out. At the same time, panelists did not always agree. The committee voted 8–7 not to include prostate cancer, for example, partly because studies have rarely linked that cancer to workplace exposures, Ward says.

And according to e-mails exchanged among committee members during the deliberations, environmental health researcher Virginia Weaver of Johns Hopkins University in Baltimore, Maryland, expressed concern about relying on *The Lancet's* firefighters study. Weaver suggested starting with a small list for now: "[R]ecommending inclusion of cancers that are most scientifically supportable ... and adding additional cancers in the future should result in more credibility for our conclusions than adding controversial cancers now," wrote Weaver, who didn't respond to a request for comment from *Science*.

Some scientists have, in fact, criticized NIOSH's decision. "There is no serious evidence that 9/11 caused anyone's cancer," says Donald Berry, a biostatistician at MD Anderson Cancer Center in Houston, Texas. Berry says that except for high-dose radiation, a

brief exposure to a carcinogen is unlikely to cause cancer. But other researchers defend the agency and its scientific advisers. Epidemiologist Graham Colditz of Washington University School of Medicine in St. Louis says that although the advisory panel recommended "more [cancers] than I would have expected," it applied "well-accepted standards" used by other bodies, such as the International Agency for Research on Cancer.

Colditz says the same methods have shaped other reports, such as that of a 1994 National Academies panel he served on that linked certain diseases in Vietnam War vets to the defoliant Agent Orange. Where scientists disagree, he says, may be on "what we mean



**Hot zone.** A scientific panel said toxic dust created on 9/11 could have contributed to 58 cancer types.

by cause." An exposure can contribute to the development of a cancer without causing the DNA damage that initiated it, he notes.

Environmental health researcher Joel Tickner of the University of Massachusetts, Lowell, says NIOSH made a reasonable policy decision. "People who served the nation in risky types of professions should receive the benefit of the doubt, because the science is always going to be uncertain," he says.

—JOCELYN KAISER



# China's Sharp Focus On Biometrics

**The country is making advances in biometrics research applications such as intelligent surveillance and infrared face recognition—and raising concerns among privacy advocates**

**SHANGHAI, CHINA**—Security cameras are ubiquitous in Chinese cities: peering along corridors, surveying busy streets, scanning public spaces. Over the past decade, the country has steadily ramped up surveillance efforts, setting a target of installing 30 million security cameras by 2015, or roughly one for every 45 people.

The sheer number of cameras dotting urban China, coupled with the limited power of civic groups to safeguard the rights of those being watched, gives privacy advocates pause. But the proliferation also poses problems for the watchers: Footage is typically reviewed manually, by police officers sitting in front of a screen searching for a specific crime, says Stan Z. Li, director of the Center for Biometrics and Security Research at the Chinese Academy of Sciences' Institute of Automation (CASIA) in Beijing. Hunting down a suspect's mug can take weeks or even months, he says: "It's very tedious. There is a huge, urgent demand for intelligent video analysis."

That's where Li and his colleagues come in. Li, who advises the central government on building an intelligent video surveillance infrastructure, has developed an application called VSearch that "makes the

impossible possible," he says. Through pattern recognition and data mining, it allows users to scan footage for people that fit certain descriptions. With closed-circuit television (CCTV) footage of a suspect wearing a red T-shirt and jeans, for example, officers can search other footage for individuals who fit the bill. VSearch works similarly for cars, searching by color and shape. "We can help the user find what he needs from a huge amount of data," Li says. "We've reduced the workload significantly."

Biometrics—a field that grew out of fingerprinting to encompass emerging areas like gait and heartbeat recognition—is in its heyday in China. A large research force, generous government support, and advances in the algorithm speeds that enable biometric applications have pushed innovation in areas like intelligent surveillance and infrared face recognition. The leading Chinese image-processing and machine-learning labs now "compete with the top institutions in the U.S.," says Anil K. Jain, a computer scientist at Michigan State University in East Lansing who sits on the advisory board of CASIA's Center for Biometrics and Security Research and makes yearly visits to Beijing to meet with Chinese counterparts. The

number of publications from China is "quite impressive," says Sébastien Marcel, an expert on pattern recognition and machine learning at Idiap Research Institute in Martigny, Switzerland.

Chinese biometric applications are being put to use for security in areas such as banking and sporting events—as they are in countries like the United Kingdom, where surveillance cameras are also ubiquitous. But critics worry that concerns about an Orwellian state—often overblown in the past—are finally well-founded. They fear that the new technologies will help China's one-party state clamp down on dissent and monitor political opponents. Technologies that allow authorities to quickly sift through vast amounts of data "really raise the specter of a Big Brother society," says Nicholas Bequelin, a researcher at Human Rights Watch in Hong Kong. In China, he adds, "there will be a sea change in the ability of the security agencies to monitor nationwide specific individuals—for law-enforcement purposes, but also for political motives."

## An unlikely catalyst

China's involvement with biometrics dates to the 1980s, when the Ministry of Public Security funded research at Tsinghua University and Peking University on fingerprint authentication, says Zhou Jie, a computer scientist at Tsinghua University in Beijing. Globally, work on biometric recognition was coming into its own. In 1987, Lawrence Sirovich and Michael Kirby, mathematicians at Brown University, discovered a way to analyze faces, essentially by turning images into vectors made up of pixels. Those processed images, called eigenfaces, paved the way for accurate face-recognition technology.

Sophisticated security equipment soon proved its worth to Chinese authorities. Footage from surveillance cameras helped security forces identify students and workers who had been in and around Tiananmen Square during the 1989 protests. The cameras had been manufactured by a U.K. company and their installation paid for by the World Bank, according to a report by the now-defunct Canadian government agency Rights & Democracy. In the wake of the crackdown, the U.S. Congress enacted export restrictions banning the transfer of crime-fighting equipment—including biometric technologies like fingerprint scanners—to China.

Those export controls sparked China's biometrics boom. Chinese researchers "had to start from scratch," Jain says, by building homegrown versions of Western technologies. China began rolling out its technologies



on a large scale in 2004, when checkpoints on the Hong Kong–mainland border installed face- and fingerprint-recognition systems for Hong Kong residents who frequently cross the border.

A series of ambitious projects followed. At the 2008 Beijing Olympics, face-recognition systems provided by the Beijing company AuthenMetric helped verify attendees at the opening and closing ceremonies, by comparing their faces to photos on their ticket application forms. The stadium hosting softball games used hand-vein recognition to control access to the stands. Today, research into China's third-generation identification cards, which will use fingerprinting, are giving the industry another boost, Zhou says.

Li sums up the advantages of working in China in one word: "People." "It's not unusual for a professor to have 20 graduate students working on face recognition," Jain says. That workforce came in handy in a venture overseen by Song-Chun Zhu, a computer scientist at the University of California, Los Angeles. Zhu developed a technology that converts images into text—another way to achieve Li's goal of making video searchable. Much of the grunt work was done at the Lotus Hill

Institute for Computer Vision and Information Science, which Zhu established in his hometown of Ezhou, Hubei Province, in 2005. He hired graduates of local art colleges to annotate images, affixing them with labels from a predetermined group of categories. The employees focused on people and cars, and just those two tasks kept them busy for years. But by 2010, the lab had built a database of 2 million images containing objects in 500 categories.

Although Zhou says China still lags behind other countries in the development and application of biometric technologies, it is now tackling problems that vex researchers around the world. Take spoofing. Standard face-recognition systems can be fooled by a person holding up a photo of another individual's face. In response to that and other challenges, Li developed infrared face recognition. The application, which was used to confirm the identity of workers at Expo 2010 Shanghai China, "has a natural immunity to photos, because it uses differ-

ent imaging-process principles," Li says. Two spectra—one visual and one infrared—make it robust, adds Marcel, who coordinates Trusted Biometrics under Spoofing Attacks, an E.U. project to combat fraud in biometric technologies. CASIA is a collaborator on the project.

Chinese researchers are also tackling a more intractable problem: "noncooperative" face recognition. While technologies like Li's allow police to search for clothing and other identifiers, pinpointing specific faces in sur-

veillance footage is still impossible. Existing recognition technologies work best in controlled, evenly lit environments like security checkpoints, where subjects are facing one direction and positioned a certain distance from the camera. That could change if researchers can develop technologies for recognizing faces from different angles, in variable lighting, and over time, as a person's appearance changes. "The most significant challenge is aging," Li says. "That's an unsolved problem."

Today, lampposts in Tiananmen Square are studded with cameras, as are the homes of political dissidents. After a series of pro-

tests in Tibet in 2008, the Chinese government installed cameras at monasteries throughout the autonomous area. Riots the following year in Xinjiang, a heavily Muslim region plagued by unrest, brought a similar response, Bequelin says: "There was a very clear policy of installing cameras to identify people and to monitor in a way that was intimidating."

The lack of citizen protections in China allows for greater latitude in employing biometric technologies. In China "you are able to cross images gathered from a CCTV system with other databases," Bequelin says: "The key here is interoperability." The Shenzhen company China Information Security Technology Inc. is

developing an identification card for migrant workers that its Web site says will be traceable through police geographical information systems. "The [privacy] issue doesn't exist in China," Jain says.

To be sure, many of the technologies being developed in China do not have sinister uses. Fingerprinting is used in some provinces to verify the identities of students taking China's annual university entrance examination. Coal mines in northeastern China use an iris-recognition system that grew out of research done at CASIA to control access and keep track of workers during an accident. "These technologies help to sustain authoritarian rule in China, but they're not only there for that," Göbel says. "They also address concerns of the population." And yet, Bequelin says, such dual-use technologies should be monitored "by a strong civil society or a political system that is responsive to citizens' concerns." In China, he adds, that "is just not there."

—MARA HVISTENDAHL



**Changing faces.** A face looks different in the visual spectrum when lighting conditions change (top row), but infrared images stay the same (bottom row).

veillance footage is still impossible. Existing recognition technologies work best in controlled, evenly lit environments like security checkpoints, where subjects are facing one direction and positioned a certain distance from the camera. That could change if researchers can develop technologies for recognizing faces from different angles, in variable lighting, and over time, as a person's appearance changes. "The most significant challenge is aging," Li says. "That's an unsolved problem."

### The specter of interoperability

In a presentation at the 2009 Asian Biometrics Consortium conference in Tokyo, Tan Tieniu, director of CASIA's National Laboratory of Pattern Recognition, said that among the sites targeted for iris-recognition systems and other biometric technologies in coming years are the country's thousands of prisons, detention houses, and labor camps—sites that house dissidents as well as prisoners. And the new flood of research

# Despite Setbacks, Optimism On Drugs for Hepatitis C

Researchers hit speed bumps on the road they hope will soon lead to a safe, short course of treatment that will rid the body of this liver-damaging virus

When a promising drug fails in clinical trials, it's usually no more surprising than a star baseball player striking out: Failure is the name of the game. But when Bristol-Myers Squibb (BMS) announced on 23 August that it was pulling the plug on a drug against hepatitis C virus (HCV) because of toxicity, disappointment reverberated throughout the field. Hopes were high for the drug: It was

The hepatitis C field was buoyed last year when the first two drugs that directly attack the virus came to market. "It's a very exciting time," says virologist Mark Sulkowski, medical director of the Viral Hepatitis Center at the Johns Hopkins University School of Medicine in Baltimore, Maryland. "We can begin to envision the possibility of making a big difference both on an individual patient level and, even more expansively, on a population level." In keeping with that therapeutic optimism, the U.S. Centers for Disease Control and Prevention last month announced a major push to identify HCV-infected baby boomers in the United States—the most affected age group—and bring them into care (*Science*, 24 August, p. 903).

Hepatitis C treatment represents a huge potential marketplace, and better drugs are badly needed. The World Health Organization estimates that HCV chronically infects 150 million people in the world. Although 15% to 25% of those infected will clear the virus without treatment, up to 20% of the chronically infected develop cirrhosis, and 1% to 5% will die from liver cancer or liver failure. The mainstay of treatment until 2011 was two drugs that have both antiviral and immune-stimulating properties. One is  $\alpha$ -interferon, a natural protein protected with polyethylene glycol ("pegylated") so it is released slowly into the bloodstream; it must be injected and has high toxicity. The second is a pill, ribavirin, that interferes with RNA metabolism. There are at least six different genotypes of HCV, and the drugs work better against some than others; genotype 1, which accounts for 60% of the infections in the world, is the hardest to treat. Genetics also has a major impact on a person's response to treatment. What's more, neither of these drugs directly attacks the virus, their mechanisms of action remain murky, they cost up to \$20,000 for a treatment course that runs from 24 to 48 weeks, and more than half the people who take them are not cured. (A cure is defined as a "sustained virological response-24," or SVR-24, in which the virus can't be detected for more than 24 weeks after treatment stops.)

The two direct-acting antiviral drugs that came to market last year both specifically cripple an HCV enzyme, protease. They must be taken with interferon and ribavirin, and although these combinations have higher cure rates—they work in roughly three of four people—they have extra toxicities and can cost up to \$50,000 per course. They are approved only for genotype 1, which is responsible for 75% of the estimated 3.2 million chronic infections in the United States, the country with the most attractive marketplace.

The BMS drug that failed in clinical trials inhibits a different enzyme, polymerase, that the virus needs to copy itself. Like several other compounds in development that target HCV polymerase, it works against all genotypes and has a high barrier to the development of drug-resistance mutations. "These drugs are difficult for the virus to evade, and that's led them to be very attractive," Sulkowski says. Researchers were hopeful that the BMS drug could be combined with other direct-acting antiviral drugs to provide a cure without the need for interferon injections. But the company scuttled the drug after serious liver and heart toxicities surfaced in a phase II clinical trial, sending nine patients to the hospital, one of whom died. The U.S. Food and Drug Administration put a "clinical hold" on a similar compound made by Idenix Pharmaceuticals also in phase II trials—and there may be a ripple effect on other drugs in development. "I think it will have an impact on how regulatory agencies evaluate the safety of all of these drugs," says David Nelson, a hepatologist at the University of Florida, Gainesville. "There's a mad rush to move forward with these all-oral drugs, rightly so, but it will lead to a rethinking."

Drugs in development have four different viral targets, and many researchers believe that hitting HCV from different angles is the best way to defeat it (see table). The polymerase inhibitors like the BMS candidate put nucleotide or nucleoside analogs into the viral RNA to cripple it, and they are known as "nukes." Clinical trials are also under way on "non-nuke" polymerase inhibitors that work by other mechanisms. New protease inhibitors are in phase II and III clinical trials, as are inhibitors of HCV's NS5A replication complex protein.

But the removal of the BMS drug and, potentially, the one made by Idenix narrows the pipeline for the nuke class. Several other promising compounds in every class have also failed in the past year. "A year ago, when all of these things were still looking good,



**First in class.** Norbert Bischofberger of Gilead says its "nuke" inhibitor has a clean record so far.

one of the furthest along in clinical trials of a new class of agents against HCV, and the Princeton, New Jersey, pharmaceutical giant had deemed it so likely to become a backbone of treatment that it paid \$2.5 billion in February to acquire the small company then developing the compound.

Researchers are now trying to understand why the drug failed and the impact it might have on other drugs in the pipeline—some of which work through similar mechanisms. Most are cautiously optimistic, however, that other drugs in development will pan out and that the failure will not seriously dent hopes of curing the disease with a short, relatively safe course of treatment that would work worldwide.



there was a lot of talk about an interferon-free, direct-acting antiviral combo succeeding, and the game is over,” says virologist Charles Rice of The Rockefeller University in New York City. “It’s amazing to me what the failure rate still is.”

One nucleoside polymerase inhibitor has moved all the way to the final phase of clinical testing, phase III, and has a clean toxicity record. Known as GS-7977, the compound was first made by Pharmasset in Princeton, New Jersey, which Gilead Sciences in Foster City, California, acquired in January for \$11.2 billion largely because of the drug’s “outstanding” early performance in human studies, says Norbert Bischofberger, Gilead’s chief scientific officer. “We felt very comfortable about the compound,” he says. (Another nucleoside included in the deal went by the wayside because it, too, had serious toxicities.)

Bischofberger suggests that there’s less concern about toxicity with GS-7977 than the BMS and Idenix nukes for several reasons. One is that GS-7977 differs in both structure and the mechanism of action from the BMS and Idenix nukes. Secondly, the Gilead drug has also been given to many more patients than the BMS one—about 2000 compared to 250. No one knows why the toxicities surfaced with the BMS drug, but Bischofberger suspects the drug might interfere with the working of mitochondria, which has occurred with some anti-HIV nukes. Mitochondria are energy factories, and both the heart and kidney are highly energy-dependent organs.

In small studies, GS-7977 combined with either ribavirin or an NS5A inhibitor made by BMS has had 100% cure rates. “Everyone hopes 7977 or one of these other nukes will be the cornerstone of an interferon-free regimen,” says Rockefeller’s Rice. Studies that combine GS-7977 with the less appealing  $\alpha$ -interferon and ribavirin are furthest along, however, and Bischofberger says the company might seek regulatory approval for that cocktail as early as mid-2013. The cocktail is being tested against all six genotypes.

Although much excitement exists about the prospects of the new era of HCV treatment, researchers stress that the many drug

combinations now being tested still might not work in some people. All the drugs in development, including GS-7977, have not been as effective in people who failed earlier on  $\alpha$ -interferon and ribavirin. It is not immediately clear why this would be so: Those older drugs don’t directly act against the virus, so using them theoretically shouldn’t create drug-resistant mutations that compromise direct-acting antiviral drugs. The Uni-

versity of Florida’s Nelson says the failure may simply flag the fact that some people have a genetic background that undermines treatment efforts.

## HEPATITIS C DRUGS IN CLINICAL TRIALS

Agent	Sponsor	Status
<b>Nucleoside/nucleotide polymerase inhibitors</b>		
GS-7977	Gilead Sciences	Phase III
BMS-986094	Bristol-Myers Squibb	Phase II
IDX184	Idenix Pharmaceuticals	Phase II
mericitabine	Hoffmann-La Roche	Phase II
<b>NS5A inhibitors</b>		
daclatasvir	Bristol-Myers Squibb	Phase III
ABT-267	Abbott Laboratories	Phase II
GS-5885	Gilead Sciences	Phase II
GSK2336805	GlaxoSmithKline	Phase II
IDX719	Idenix Pharmaceuticals	Phase I/II
<b>Protease inhibitors</b>		
asunaprevir	Bristol-Myers Squibb	Phase III
BI 201335	Boehringer Ingelheim	Phase III
simeprevir	Janssen/Tibotec/Medivir	Phase III
vaniprevir	Merck	Phase III
ABT-450/r	Abbott Laboratories	Phase II
ACH-1625	Achillion Pharmaceuticals	Phase II
danoprevir/r	Hoffmann-La Roche/Genentech	Phase II
GS-9256 & GS-9451	Gilead Sciences	Phase II
MK-5172	Merck	Phase II
<b>Non-nucleoside polymerase inhibitors</b>		
ABT-072 & ABT-333	Abbott Laboratories	Phase II
BI 207127	Boehringer Ingelheim	Phase II
BMS-791325	Bristol-Myers Squibb	Phase II
setrobuvir	Anadys/Hoffmann-La Roche	Phase II
tegobuvir	Gilead Sciences	Phase II
VX-222	Vertex Pharmaceuticals	Phase II

versity of Florida’s Nelson says the failure may simply flag the fact that some people have a genetic background that undermines treatment efforts.

Sulkowski of Johns Hopkins and colleagues revealed in the 17 September 2009 issue of *Nature* that genetic variations near a gene known as *IL28B* determined who best responded to the  $\alpha$ -interferon/ribavirin regimen. Specifically, the *IL28B* CC genotype found in European populations had about a

twofold higher success rate with treatment than the *IL28B* TT genotype commonly found in African-American and Hispanic populations (*Science*, 29 October 2010, p. 579). Nelson says the *IL28B* TT genotype might be a marker that people have compromised innate immune responses. “You need innate immunity to control removing hepatitis C from the cells,” Nelson says. “Non-

responders who have the *IL28B* TT allele have a different innate immunity in the liver that probably raises the barrier to what drugs you’ll need.” Gilead’s Bischofberger suspects that the problem might not be so intractable. He says  $\alpha$ -interferon and ribavirin may create non-responders by, say, leading HCV out of the liver and into another part of the body that the direct-acting drugs have more trouble reaching. He says studies under way that treat naïve populations with only direct-acting antiviral drugs will reveal whether non-responders are a genetic phenomenon or a consequence of  $\alpha$ -interferon/ribavirin treatment.

Current treatments work best in people during early stages of disease, and Rockefeller’s Rice worries that the new drugs similarly may work well in people with mild liver disease but not in those with cirrhosis. “People in the greatest need of being treated and cured have the poorest response rates, and they aren’t the front runners for early-stage clinical trials so there’s not as much data for how these drugs are going to work in these populations,” Rice says.

Still, in spite of the failure of the BMS drug, the future looks so bright for HCV treatment that many clinicians are encouraging infected patients to wait to start treatment until the next crop of drugs comes to market that are more effective, safer, and require shorter times to

bring about a cure. “Clearly, patients with milder liver disease have reason to wait,” says Nelson, who expects several powerful new regimens on the market within 3 to 5 years. Rice is more cautious. “We shouldn’t say the game is over until it’s over,” he says, borrowing a line from baseball legend Yogi Berra. “We need a good stable of these compounds, and it’s probably going to take a while to get there.”

—JON COHEN

## LETTERS

edited by Jennifer Sills

## Friends in Fungi

ALTHOUGH FUNGI ARE A GREATER THREAT TO CROPS and forests than ever before ("Attack of the clones," K. Kupferschmidt, *News Focus*, 10 August, p. 636), we should not expel them completely. Mycorrhizal fungi are ancient and indispensable plant root mutualists (1, 2) that provide not only vital nutrients to their plant hosts (about 80% of plant species) (3) but also such services as drought resistance (4), heavy metal uptake (5), and pathogen protection (6). Mycorrhizal fungi even protect plants from some emerging fungal diseases (6).

Mycorrhizae absorb soil minerals and exchange them for host carbon (3), allowing plants to survive in relatively nutrient-poor land soils (1). The symbiosis acts as a major carbon sink, with plants allocating up to 20% of their photosynthate to these fungi (3). Mycorrhizal fungi are thus key drivers of global carbon and nutrient cycles. These symbionts, which are found in association with most major crops (7), are a key resource for developing a more sustainable agriculture. In a world facing rapid depletion of phosphorus fertilizer (8), appropriate management of mycorrhizal fungi could potentially save fertilizer and increase yields (9).



Mycorrhizal fungus.

Our fight against emerging fungal plant diseases should be strong and powerful. However, we must proceed with caution. We should not blacklist all fungi, but rather enlist those on our side. Our strategy should avoid broad-spectrum fungicides, such as propiconazole and fenpropimorph (10). We cannot afford to generalize all fungi as threats; we must learn to know our enemies from our friends.

GIJSBERT D. A. WERNER\* AND E. TOBY KIERS

Institute of Ecological Science, VU University Amsterdam, 1081 HV Amsterdam, Netherlands.

\*To whom correspondence should be addressed. E-mail: g.d.a.werner@vu.nl

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## Political Science in Peril

RECENTLY, THE U.S. HOUSE OF REPRESENTATIVES passed an amendment, proposed by Congressman Jeff Flake (R-AZ), that would allow Congress to intervene in the National Science Foundation's (NSF's) merit review process (1). Flake's amendment would prevent NSF scientists from evaluating and supporting scientific studies of politics and government. This is a bad idea.

The program targeted by Flake supports

research performed by scientists with a range of backgrounds, including statistics, applied mathematics, the neurosciences, economics, genetics, and psychology. The political science program has supported five Nobel laureates and has produced thousands of controlled experiments, precise definitions, accurate measurements, computer models, and other means of clarifying causes of what has happened in the past and the effects of organizational decisions on people's lives.

For example, the program supports experiments that differentiate seemingly plausible tactics for recovering after natural disasters from strategies that produce longer-lasting results. Another study identifies words and domestic commitments that change treaties from meaningless symbols to instruments for peace. Yet another project develops better measures of what citizens see and want, which can help governments base policy on need rather than "spin." Many other studies clarify the inner workings of other countries, which

helps diplomacy and international trade.

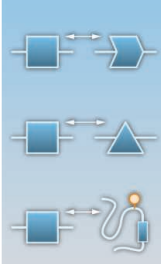
In debates on the Flake amendment, some have claimed that you do not need science to explain government and politics. That is partially correct. Americans explain these topics in many different ways. Journalists put distant events into story form and make governmental and political phenomena easier for people to visualize. These stories energize democracy. But many storytellers are not interested in objective evaluations of their views. Scientists, by contrast, often expect to be judged by the detail and replicability of their explanations. When people's lives and livelihoods are at stake, it is not enough to spin a good yarn. Societies benefit from being able to differentiate false stories from an explanation that is consistent with logic and the best available evidence. Supporting a science that informs government and policy is critical to any modern society that wishes to become or remain effective and efficient.

Fortunately, a recently passed Continuing

## Letters to the Editor

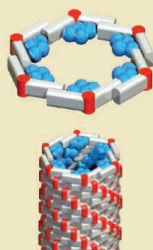
Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to [www.submit2science.org](http://www.submit2science.org).





Protein disorder

1460



Shrink, swell, repeat

1462

Resolution sustains NSF's current programs and has given the research community a bit of a respite. The next few months provide an opportunity for better minds to continue to support NSF's many contributions to our nation's scientific infrastructure. When the time for a new budget comes, the United States Congress will have an opportunity to follow the example set by decades of its foresighted predecessors from both parties. At that time, it should allow NSF to continue using its world-respected peer-review processes to determine when and how science can best inform America and the world about key aspects of policy and governance.

ARTHUR LUPIA

Institute for Social Research, University of Michigan, Ann Arbor, MI 48106, USA. E-mail: lupia@umich.edu

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#### TECHNICAL COMMENT ABSTRACTS

### Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

James E. Cresswell and Helen M. Thompson

Henry *et al.* (Reports, 20 April, p. 348) used a model to predict that colony collapse in honey bees could be precipitated by pesticide-induced intoxication that disrupts navigation. Here, we show that collapse disappears when the model is recalculated with parameter values appropriate to the season when most pesticide-treated flowering crops bloom.

Full text at [www.sciencemag.org/cgi/content/full/337/6101/1453-b](http://www.sciencemag.org/cgi/content/full/337/6101/1453-b)

### Response to Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

Mickaël Henry, Maxime Béguin, Fabrice Requier, Orianne Rollin, Jean-François Odoux, Pierrick Aupinel, Jean Aptel, Sylvie Tchamitchian, Axel Decourtye

Cresswell and Thompson have suggested an elegant way to improve honey bee colony simulations when forecasting the fate of colonies exposed to pesticides. Following their recommendations, we rescaled the model on a sound empirical data set. The adjusted forecast is bleaker than their tentative scenario.

Full text at [www.sciencemag.org/cgi/content/full/337/6101/1453-c](http://www.sciencemag.org/cgi/content/full/337/6101/1453-c)

#### CORRECTIONS AND CLARIFICATIONS

**Reports:** "Shear-activated nanotherapeutics for drug targeting to obstructed blood vessels" by N. Korin *et al.* (10 August, p. 738). The Report was published online on 5 July 2012, not 28 June 2012 as indicated. The HTML and PDF versions online have been corrected.

**News Focus:** "The ingredients for a 4000-year-old proto-curry" by A. Lawler (20 July, p. 288). In the third paragraph, the article refers to Washington State University. WSU is in Vancouver, WA, not Vancouver, BC. This has been corrected in both the HTML and PDF versions online.



theBUZZ

## Honorary Authorship

In their 31 August Editorial (p. 1019), P. Greenland and P. B. Fontanarosa called on researchers to put an end to honorary authorship. Honorary authorship remains common; researchers add the names of prominent scientists to boost their paper's credibility, and senior scientists demand that their names be added to the work of younger researchers. Greenland and Fontanarosa assert that adding authors who did not contribute directly is fraudulent, and they urge journals, research institutions, and senior scientists to address the problem. Readers wrote in to add their perspectives, many with their own experiences of being pressured to add authors to their work. Excerpts from some of these comments are below. You can read all the comments at <http://comments.sciencemag.org/content/10.1126/science.1224988>.

#### A selection of your thoughts:

...[A]sking all authors to take credit for the whole of the work is potentially problematic and might dampen willingness to collaborate. Taking credit for what you have contributed and being willing and aware of the entire content of a paper might be a reasonable compromise....

—Jim Woodgett

...Since they are named on the grant, most PIs and co-investigators will want their names on project papers regardless of whether they have contributed to the published work or not....

—Nick Riviera

...If you want this to work, journals should remove author names and affiliations while sending papers for review....

—Ram Subramanian

The final paragraph [of the Editorial] suggests that it will be the senior scientists that will set an example for the younger generation. I suspect it will be the opposite, that our students will learn how to do it right despite us. As the wise man said, "Science advances funeral by funeral."

—David Barnert

...At what point should a PI be dropped from the author list? They are, after all, usually responsible for the whole research project, even if the actual number of conversations held with the first author is minimal. Should a PI who becomes essentially a manager and behind-the-scenes... advocate for the science of others never be author of a paper?...

—Julia Hargreaves

...[T]he community must close existing loopholes in academic authorship standards, such as...research projects [that] share their data only with researchers who agree to add the respective consortium to the author list of published papers using these data.... [T]hese groups declare that by including a footnote in which they renounce authorship, they are merely claiming credit as non-author contributors....Future authorship standards should, therefore, clearly state that only authors may be listed on the author byline....At the same time, incentive systems for contributions such as data or software should be created to reduce the perceived need for quid pro quo authorships. Researchers who provide resources to the community should be able to list these contributions in their résumés, and equal consideration should be given to these and traditional publications in funding and promotion decisions.

—Torsten Rohlfing

# Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

James E. Cresswell<sup>1,2\*</sup> and Helen M. Thompson<sup>3</sup>

Henry *et al.* (Reports, 20 April, p. 348) used a model to predict that colony collapse in honey bees could be precipitated by pesticide-induced intoxication that disrupts navigation. Here, we show that collapse disappears when the model is recalculated with parameter values appropriate to the season when most pesticide-treated flowering crops bloom.

Systemic neonicotinoids, such as thiamethoxam and imidacloprid, are currently among the most widely used insecticides in crop protection (1). Neonicotinoids are applied as foliar sprays or seed dressings, and the chemical pervades the plant systemically to protect it against insect pests (2). Honey bees (*Apis mellifera*) ingest residues of these pesticides when they consume nectar and pollen from neonicotinoid-treated flowering crops (3), and there is concern that this may contribute to colony collapse because the neonicotinoids are neurotoxic to insects (4). Consequently, it is important that pesticide regulators assess the risks that dietary pesticides pose to honey bees.

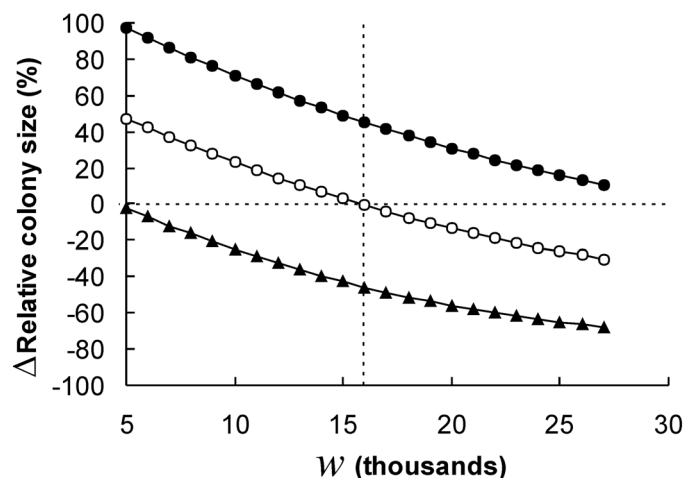
Laboratory trials have shown that doses of dietary neonicotinoid at trace levels (up to 10 parts per billion) are capable of harming individual honey bees at sublethal levels (5). For example, dietary exposure to trace dietary imidacloprid is expected to reduce behavioral performance in adult honey bees by between 6 and 20% (6). However, in a social insect like the honey bee, the ecologically relevant entity is the colony, not the individual. Therefore, it is important for risk assessors to establish whether the effects observed on individuals in the laboratory translate into impacts on colonies.

No published field experiment has yet had sufficient statistical power to detect effects on colonies of the magnitude observed on individual bees in the laboratory (6). When decisive experiments are unavailable, scientists may instead make forecasts with models and computer simulations. This approach has been used in the case of global climate change (7), and the results have the potential to be highly influential among policy-makers and regulators. In their recent paper, Henry *et al.* (8) used this approach and

predicted impact on honey bee colonies of dietary thiamethoxam. Specifically, Henry *et al.* (8) solved a model of colony dynamics (9) and concluded that collapse would be precipitated because pesticide-induced intoxication disrupts navigation and foragers fail to return home. Here, we show that the prediction may be inaccurate when more environmentally relevant parameter values are used.

Henry *et al.* populated virtually all parameters in their model with empirically based values except one, namely  $w$ . Parameter  $w$  moderates the maximum daily rate of production of new workers,  $L$ , so that it has a density-dependent sigmoidal response:  $L \times N / (N + w)$ , where  $N$  is the number of adult bees in the colony. Thus,  $w$  represents the colony size at which new workers are produced at half the maximum rate. The originators of the model (9) appear to have chosen a value of  $w$  to generate model outputs that fit observations of the average age of onset of foraging by adult bees and their overall life span (10).

**Fig. 1.** The change in size of a honey bee colony over 30 days (y axis) relative to the parameter that governs the rate of production of new adult workers,  $w$  (x axis), for three scenarios of pesticide exposure. Upper curve (closed circles), background mortality with no additional mortality due to pesticides; middle curve (open circles), background mortality with additional mortality due to pesticide-induced navigation failure in a familiar landscape; lower curve (closed triangles), background mortality with additional mortality due to pesticide-induced navigation failure in an unfamiliar landscape. Horizontal dashed line indicates zero colony growth; vertical dashed line indicates the value of  $w = 16,000$ . In these solutions, the initial size of the colony is  $N = 18,000$  adults, but  $N = 15,000$  produces virtually identical results. Other parameter values are as given in Henry *et al.* (1).



The model's output is very sensitive to the value of  $w$  (Fig. 1). Like the model's originators (9), Henry *et al.* assumed that  $w = 27,000$  (8), but this is unrealistic because a colony of 18,000 adult bees (8) then grows only by 11% in a month in the absence of pesticide (Fig. 1). In spring or early summer, which is when bees in Europe are typically exposed to neonicotinoid-treated mass-flowering crops such as oilseed rape (*Brassica napus*) (8, 11), a colony of this size can increase by >40% over 30 days (12, 13), which is consistent with  $w \approx 16,000$  (Fig. 1). Indeed, using  $w = 16,000$  in the model very accurately predicts observations of adult life span on similarly sized colonies in the absence of pesticide (10). Specifically, we find a very good correspondence between model and observations (10) in both the average age of onset of foraging by bees (model = 17.8 days versus observed = 17.7 to 19.4) and overall adult life span (model = 24.3 days versus observed = 22.3 to 22.8). Thus, our value for  $w$  is at least as plausible as that used by Henry *et al.* We speculate that Henry *et al.*'s  $w = 27,000$  may be more appropriate for forecasting pesticide effects on a slow-growing colony, perhaps in autumn.

When we recalculate the model using  $w = 16,000$  and with pesticide-induced loss of foragers at the rates measured by Henry *et al.*, pesticide exposure severely reduces colony size only if the intoxicated workers navigate an unfamiliar landscape (Fig. 1). However, the experimental doses of the neonicotinoid pesticide thiamethoxam administered by Henry *et al.* are daily totals, which would seem to assume that bees forage from a treated crop repeatedly throughout the day. The experimental doses are therefore appropriate only to bees operating in a familiar landscape. From this interpretation, the model predicts that a month of pesticide exposure leaves

<sup>1</sup>Biosciences, College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK. <sup>2</sup>Centre for Pollination Studies, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India. <sup>3</sup>The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK.

\*To whom correspondence should be addressed. E-mail: j.e.cresswell@ex.ac.uk



colony size virtually unchanged (Fig. 1) and would not precipitate colony collapse.

As Henry *et al.*'s experiments so elegantly demonstrate, there is no question that dietary thiamethoxam harms honey bee colonies by elevating the mortality of adult foragers through navigation failure, at least when the entire daily intake of a forager is consumed in a single dose. However, what is at issue is whether thiamethoxam is capable of causing colony collapse. Our results suggest that dietary thiamethoxam would not precipitate collapse in healthy colonies in spring, but this does not rule out the possibility that colonies will be more vulnerable later in the year when their capacity to replace lost workers has diminished. Based on our analysis, we argue that (i) the forecast impact of thiamethoxam on honey bees is nuanced, being highly contingent on colonies' capacity for producing workers; (ii) pesticide regulators should be cautious in using

this model's outcomes when formulating a stance on controlling the future use of thiamethoxam; and (iii) colony-growth models may have a very important role in future risk assessments, but further research is required to ensure that they are fully validated and appropriately configured for the environmentally relevant context in which they are to be applied.

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# Response to Comment on “A Common Pesticide Decreases Foraging Success and Survival in Honey Bees”

Mickaël Henry,<sup>1,2\*</sup> Maxime Béguin,<sup>2,3</sup> Fabrice Requier,<sup>4,5</sup> Oriane Rollin,<sup>2,6</sup> Jean-François Odoux,<sup>5</sup> Pierrick Aupinel,<sup>5</sup> Jean Aptel,<sup>1,2</sup> Sylvie Tchamitchian,<sup>1,2</sup> Axel Decourtye<sup>2,6</sup>

Cresswell and Thompson have suggested an elegant way to improve honey bee colony simulations when forecasting the fate of colonies exposed to pesticides. Following their recommendations, we rescaled the model on a sound empirical data set. The adjusted forecast is bleaker than their tentative scenario.

Henry *et al.* (1) reported that sublethal doses of thiamethoxam, a neonicotinoid pesticide used on some common flowering crops, reduce the ability of exposed foraging honey bees to find the way back to their colony. The daily mortality probability due to homing failure, termed  $m_{hf}$ , was estimated to lie somewhere between 0.102 and 0.316 for foragers exposed and released 1 km away from their colony. Honey bee population models (2) predict that this abnormal mortality level causes a major deviation from the expected demographic trajectory. Models were run with 1-month exposure durations, with the underlying idea to simulate colonies exposed to treated oilseed rape.

Cresswell and Thompson (3) have proposed an adjustment of the population model and found no population change over this duration, at least with the least pessimistic exposure scenario ( $m_{hf} = 0.102$ ). Specifically, they have suggested an elegant way to assess  $w$ , the only model parameter that could not be calculated from empirical data. Parameter  $w$  is a negative feedback constant that moderates the production rate of new workers as the colony matures. Cresswell and Thompson cleverly used empirical colony growth data to infer  $w$ . They assumed that a colony of 18,000 individuals may grow by 40% in a month during oilseed rape blooming period (4), which is reached with  $w = 16,000$ . When this analytical solution is transposed to the exposure scenario, no population change is detected [figure 1 in (3)]. Cresswell and Thompson

noted that our parameterization ( $w = 27,000$ ) assumed an 11% growth only in the absence of pesticides and therefore predicts an excessive decrease for exposed colonies (–30% with  $m_{hf} = 0.102$ ).

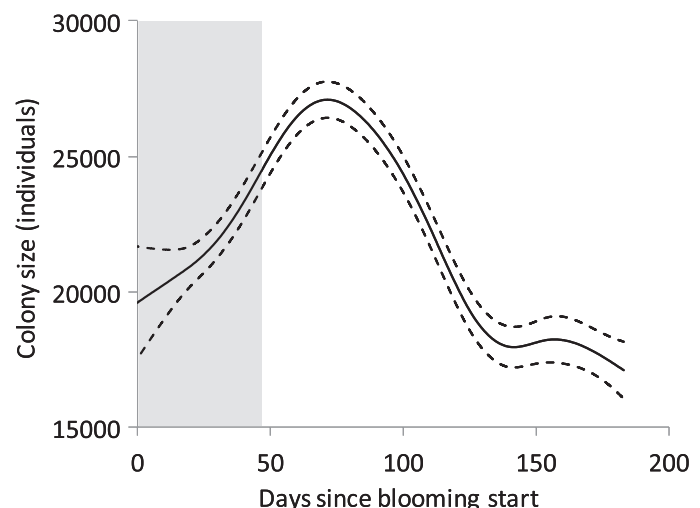
The technical comment by Cresswell and Thompson is a sound cautionary note about simulation-based risk assessment of nonintentional pesticide effects. However, we would like to rectify an inaccurate statement in their comment. We did not claim that our simulation outcome had predicted colony collapse due to homing failure. Instead, we concluded that the levels of homing failure we measured are high enough to cause “a major deviation from the expected dynamic” (1). This conclusion is not ruled out by Cresswell and Thompson’s model adjustment, as is merely illustrated by the virtually constant colony size gap (a ~45% difference) between exposed and nonexposed scenarios, regardless of the chosen  $w$  value [figure 1 in (3)]. Furthermore, we believe that the tentative value of 40% for colony growth on which Cresswell and Thompson have based their reasoning is not robust. It seems that it was obtained from three

monitored colonies only, and no indication is given on the use of oilseed rape in the vicinity (4). Given the tremendous variability one usually observes among colonies, any attempt to derive model parameters from empirical data deserves stronger support. Here, we followed Cresswell and Thompson’s valuable suggestion to solve the calculation of  $w$ , using a strong empirical data set.

We reanalyzed the ECOBEE (Ecological Honeybee Colony Monitoring) data set used in our original study to set a range of realistic starting values for colony size ( $I$ ). ECOBEE is managed by our research groups with the objective to provide ecologists with detailed honey bee colony dynamic data under real beekeeping management conditions. Colony monitoring data, including adult population size, are collected biweekly within a network of about 50 colonies per year. Over the 2008 to 2011 beekeeping seasons—i.e., before thiamethoxam was marketed for oilseed rape protection in our study area—a total of 208 colonies have been monitored. They were allocated into 40 apiaries, evenly distributed over the 450-km<sup>2</sup> study area in order to cover a wide range of landscape contexts. As explained in our original study, this territory is an intensive cereal farming system where oilseed rape accounts for 8 to 10% of total land cover.

We computed an empirical colony size model to derive real colony growth data and to recompute exposure simulations accordingly. Colony size was modeled using generalized additive mixed models (GAMM). This modeling technique allows adjusting a temporal spline based on maximum likelihood, while giving the possibility to account for repeated measurements on the same colonies within a given year. The temporal axis was scaled on the Julian date since the beginning of oilseed rape blooming. Blooming dates are available from local long-term phenological surveys (5). The temporal spline predicts a steep population growth encompassing the blooming period, and a gradual decline thereafter, as colonies expend less effort into reproduction and

**Fig. 1.** Empirical honey bee colony size data ( $\pm 1$  SE) used to recalculate population models. Colony size is measured biweekly by weighting all hive elements (comb, frames, and supers) with and without bees, as a part of the ECOBEE monitoring facility. Seasonal changes in colony size are modeled by a temporal spline (GAMM). The shaded area denotes oilseed rape blooming period.



<sup>1</sup>INRA (Institut National de la Recherche Agronomique), UR406 Abeilles et Environnement, F-84914 Avignon, France. <sup>2</sup>UMT Protection des Abeilles dans l’Environnement, Site Agroparc, F-84914 Avignon, France. <sup>3</sup>Association pour le Développement de l’Apiculture Provençale, F-13626 Aix-en-Provence, France. <sup>4</sup>Centre d’Etudes Biologiques de Chizé, CNRS (USC-INRA 1339), UPR1934, F-79360 Beauvoir-sur-Niort, France. <sup>5</sup>INRA, UE1255, UE Entomologie, F-17700 Surgères, France. <sup>6</sup>Association de Coordination Technique Agricole, Site Agroparc, F-84914 Avignon, France.

\*To whom correspondence should be addressed. E-mail: mickael.henry@avignon.inra.fr



more into food storage (Fig. 1). Although the oilseed rape blooming period hardly lasts more than 1 month in a particular field, there is a substantial temporal lag among fields and phenotypes, so that blooming period covered on average 48 days at the territory scale during the 2009 to 2011 ECOBEE monitoring program. To pinpoint the most plausible range of values for  $w$ , we sought for the analytical solutions that matched the average colony size values observed during different 30-day periods: (i) the initial blooming period (days 0 to 30 on the temporal axis, 11.7% growth), (ii) the full blooming period (days 10 to 40, 15.0%), and (iii) the late blooming period encompassing the steepest population growth (days 18 to 48, 18.7%). Analytical solutions for  $w$  were 24,932, 22,880, and 20,886, respectively, and also returned a close correspondence between observed and theoretical average age of onset of foraging (observed = 17.7 to 19.4 days, model = 18.2 days) and overall adult life span (observed = 22.3 to 22.8, model = 24.6). When homing failure was set to  $m_{hf} = 0.102$ , predicted population changes were  $-28.6\%$ ,  $-25.5\%$ , and  $-22.1\%$ , respectively.

These empirical-based scenarios are more pessimistic than the steady colony state predicted by Cresswell and Thompson. However, we agree that substantial improvement is needed before one could use honey bee colony modeling in its current form for risk assessment. We initially used modeling as a tool to get estimates of what observations made at the individual level would imply for the colony as a whole. Sound model adjustments have been proposed (3), but further issues remain to be documented to gain accuracy. Among others, homing failure should be re-evaluated with regard to (i) doses matching in-hive exposures of conspecifics and larvae by contaminated pollen and honey (6) and (ii) acute versus chronic exposure regimes at the foragers' life scale. The latter aspect, in particular, is still an unsolved debate. It is currently unclear whether acute experimental exposures overestimate sublethal effects compared with chronic regimes (7). Likewise, population modelers should consider trying different values of the egg-laying rate, which appears to follow a sharp decline after oilseed rape blooming, as well as the post-exposure homing distance foraging honey bees need to cover. Those

two parameters are expected to be largely influential in the procedure.

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## TECHNOLOGY

# The Social Stakes of Interoperability

Laura DeNardis

Anyone with a box full of tangled, incompatible device chargers understands that interoperability is not a given. Buying a digital book can create portability constraints such as restrictions on viewing the purchased item on different devices. Moving content from one social media platform to another can pose an intractable problem. An entire generation stores its pictures, video, and music in the cloud on private platforms such as Flickr and YouTube. The history of information technology is replete with once-popular companies being supplanted by newer platforms, so the obvious question is whether today's digital culture, archives, and libraries will be accessible to future generations.

The stakes have never been higher for interoperability, a topic taken up by John Palfrey and Urs Gasser in *Interop: The Promise and Perils of Highly Interconnected Systems*. The ability for systems to interconnect is closely related to efficiency. Economic arguments suggest that advancements in interoperability increase competition and innovation. Think of the 19th-century development of a standard gauge interconnecting railroads across the vast expanse of North America or, more than a century later, the standardization that allows us to exchange e-mail across different types of applications and hardware devices. Not surprisingly, companies sometimes prefer proprietary approaches that limit interoperability and lock in users for competitive advantage. But consumers usually want technological systems to be able to seamlessly exchange information.

The authors (specialists in information law) presciently use real-world examples to remind us that "this growing level of interconnectedness comes at an increasingly high price." A Faustian bargain for interoperability can threaten indi-

vidual privacy and security, whether compromised by an acquaintance, government, corporation, or hacker. Facebook's Beacon product (2007–2009), which connected individual accounts to third-party retail sites, was an example of "interoperability gone awry." When a Facebook user purchased something on one of these partner websites, a notice about the product would be automatically generated on the member's personal page (and possibly appear in the news feeds of the user's friends). More recently, the hacking of Sony's PlayStation gaming network potentially exposed the personal account information of its millions of users. Interoperability does not cause these privacy and security concerns, but it is an enabling factor.

When interoperability standards enter widespread usage, they can also lock society into less-efficient technologies in the same way that proprietary standards can lock users into a single company's vertical line of products. The majority of keyboards, even those on tablets and smart phones, still use the Charles Dickens-era QWERTY layout originally designed to prevent adjacent typewriter keys from mechanically jamming

by dispersing the keys most likely to be typed in succession. Superior alternatives designed to enable much faster typing have not gained traction because of the conservative momentum of such a socially entrenched standard.

Palfrey and Gasser emphasize finding the optimal rather than maximum level of interoperability. They also explore how to determine the appropriate balance between markets and governments for achieving this optimal level. States, at a minimum, create background rules for how companies interoperate in a variety of contexts (e.g., enforcing privacy policies in industries such as health care and finance). The problem then becomes one of legal interoperability, as companies that provide global services must navigate the varied waters of different countries' laws.

Achieving the appropriate degrees of technical and legal interoperability will be even more critical in the future as society moves information further into borderless worlds such as smart-grid energy infrastructures, cloud computing, and eHealth.

Most societies view interoperability as a partial solution to the enormous costs associated with health care systems in which the lack of standardization prevents the efficient and life-saving exchange of patient information among health care providers, insurance companies, and medical labs. *Interop* underlines both the social need for greater digitization and interconnectivity in this complex area

of information technology and the possibly problematic implications of greater interoperability for individual privacy, health care liability, and security. The authors develop an interesting parallel between interoperability's role in solving health care problems and its dramatic potential for improving energy delivery efficiency through smart-grid infrastructures in which interconnected networks sense, measure, and deliver energy in response to changing conditions.

The common thread of a need for greater interoperability ties together some of the most important public policy issues of our time.

## Interop The Promise and Perils of Highly Interconnected Systems

by John Palfrey  
and Urs Gasser

Basic Books (Perseus),  
New York, 2012. 304 pp.  
\$28.99, C\$33.50.  
ISBN 9780465021970.



**The golden spike.** Palfrey and Gasser describe the May 1869 joining of the rails of the Union Pacific and Central Pacific railroads at Promontory Summit, Utah, as "a major event in the history of interoperability."

The reviewer is at the School of Communication, American University, 4400 Massachusetts Avenue NW, Washington, DC 20016, USA. E-mail: denardis@american.edu



Rather than reifying interoperability as a social value in itself that should be pursued at all costs, we should view it as a mechanism for achieving specific social and economic objectives in these crucial policy areas. *Interop* will serve as a constructive and motivating resource for policymakers, citizens, and practitioners interested in the outcome of emerging, hyperconnected areas such as smart-grid energy infrastructures, cloud computing, and eHealth systems or in ensuring our ability to preserve digitally stored culture and knowledge for generations to come. As the authors themselves argue, “We, as societies, must take interop seriously as we hurtle into a future full of increasingly complex and interconnected systems.”

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## ENVIRONMENT

# Nature—Half Lost or Half Saved?

Douglas McCauley

Half of the world's rainforests have been felled. Wild tiger numbers have been reduced by 95%. Climate change and ocean acidification threaten to make it impossible for coral reefs to persist beyond the year 2050. Andrew Balmford says: good news—50% of our rainforests remain, there are still a few tigers roaming about, and our reefs haven't dissolved, yet.

Social revolutions emerge out of the cacophonous dialectic of pessimists and optimists. Perhaps nowhere is this more evident than in the writing of authors motivating the movement for nature conservation. Eco-catastrophist authors are conspicuous and abundant. The titles alone from the classics in this genre are enough to make us shiver: *Population Bomb*, *The End of Nature*, *Requiem for Nature* (1–3). While gloomy, these texts have done the important service of publicizing the dire position we have put our environment in. Such reads, however, can leave one slightly more inclined to jump off a cliff in the Grand Canyon than to join a campaign to save the endangered condors roosting on those cliffs. As counterpoint to the tenor of these texts, Balm-

ford offers up *Wild Hope*, a collection of conservation success stories intended to infuse new optimism into the science and practice of nature conservation.

The case studies Balmford (a conservation biologist at the University of Cambridge) writes about are as much about people as they are about ecosystems. *Wild Hope*'s heroes of conservation come in diverse forms. They are tanned surfers fighting invasive plants, bright-eyed rhino guards, and burly foresters safeguarding endangered woodpeckers. The conservation tools these characters employ are as diverse as they are: hybrid poverty relief and native species protection programs, ecosystem service payments, 0.315 rifles, crafty political maneuvering, and aircraft-aided drops of poison sausages. Conservation advances, or at least fails to lose ground, through the dedicated service of Balmford's motley eco-crew.

For the unreachable environmental pessimist, there is yet material sufficient to glean perverse critical enjoyment from *Wild Hope*. His “best of” conservation stories include a celebration of efforts to clean up patches of forest that were clear-cut for strip mining and the successful conversion of a dammed bay ecosystem into grassland habitat. There are clearly some dregs in Balmford's half-full cup. Its contents are also, at times, a little hard to swallow. We commend the cutting down of forests (invasive trees) in South Africa and then demonize forest cutting (native trees) in Ecuador—both actions

that ostensibly protect water provisioning, although scientific support for such benefits is still being amassed. Balmford, however, generally does not tiptoe around these shortcomings and inconsistencies. He asks hard questions of his heroes and saddles us with the frank complexities that characterize the practice of on-the-ground conservation. This attempt to maintain balance makes *Wild Hope* a more palatable, instructive, and genuinely inspiring read than other conservation science texts also written in rose-colored ink.

Although in places the author's enthusiasm to inspire becomes a bit too saccharine (replete with quotes from Helen Keller and Teddy Roosevelt), it is difficult not to be reinvigorated with the possibility that there is indeed hope for the survival of important remaining parts of our natural world. Primed for action as I approached the end of the book, I was left asking only: How do I get started? And in the last chapter, Balmford lays out



**Rehabilitated mines.** After removing bauxite at its lease in Western Australia, Alcoa goes to great lengths to restore the jarrah forest.

seven tenets for achieving success in conservation: be a leader; be patient; be bold; do careful and relevant research; be creative; be politically savvy; and strive for improvement, not perfection. All true, yet the banality of these suggestions (which read like a proselytical cross between an Obama speech and the *Bhagavad Gita*) left me a bit intellectually undernourished. I wanted more depth from the opus vitae of a scholar who has contributed to more than ten articles on the science of conservation in *Science* alone and countless others elsewhere.

Perhaps, however, there is a kind of genius in *Wild Hope*'s opacity and simplicity that sets it apart from bibliographic congeners in the genre of conservation writing. Science plays a critically important role in framing the dialogue for how and where we manage ecosystems. Balmford makes this clear. However, interspersed between the book's lines one finds the suggestion by this erudite scholar that nature is ultimately not saved by learned scholarship but by people doing hard yet simple things with rifles, greenhouses, and conscientious grocery lists. *Wild Hope* provides neither an easy nor a hard solution for best safeguarding biodiversity. Conservation, we learn, succeeds by doing just what works. Balmford's vague, but sincerely impassioned, response makes more neurons fire in the region of the heart than in the head. Nonetheless, perhaps that is just the muscle needed for protecting nature.

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# Global Indicators and Targets for Noncommunicable Diseases

S. Y. Angell,\* I. Danel, K. M. DeCock

Measurable, ambitious, and achievable indicators with targets are critical, but political commitment to achieve them is the main determinant of outcomes.

In September 2011, the United Nations (UN) convened a High Level Meeting (HLM) of member states to address a largely neglected, global reality: Noncommunicable diseases (NCDs)—including heart disease, stroke, cancer, diabetes, and chronic lung diseases—kill more people than other causes, health and non-health related, and the world is ill-prepared to respond. This was only the second such UN meeting of heads of state focused on a health issue, the first having been on HIV/AIDS in 2001. Without more effective and focused action, the growing burden of NCDs threatens to undermine increasingly interdependent development and economic agendas (1–3). The 2011 meeting ushered in the potential for an orchestrated response, facilitated by a mandate that the World Health Organization (WHO), in consultation with member states, develop a global monitoring framework with key indicators and targets to be achieved by 2025.

The task is to be completed by the end of 2012 (1). Only one global voluntary indicator with a target has received formal member-state endorsement thus far: reduce the probability of premature mortality from NCDs by 25% by 2025. Another 10 indicators with targets, and 9 indicators without targets, are proposed and under development (2), with the deadline just months away.

Although we focus on technical aspects, it is important to note that they are just one of the determining factors of the national and international processes of NCD indicator selection and target setting. Through member-state agreement, the WHO process is informed by a diversity of stakeholder interests, from public to private and civil society, as well as by foreign policy and economics. Sustainable changes in NCD outcomes will demand a whole-of-government and multisectoral engagement, and global targets have implications for foreign assistance. All of these factors make the process of goal setting both political and scientific.

## Technical Criteria and Selection Process

The creation of indicators, measurable characteristics that describe an aspect of health, and targets, specific time-bound changes in indicators expected to be achieved, is not new to global health and development (4). In addition to providing an objective means to assess progress and impact, indicators and targets express a collective commitment to act, which can spark coordinated action and the mobilization of resources (4, 5). For example, a 1958 World Health Assembly (WHA) resolution to eradicate small pox globally was followed by a ratified plan and budget 10 years later, and eradication of the disease by 1979. The UN Millennium Development Goals (MDGs), adopted in 2000, are credited for increased funding (6) and likely contributed to the large observed reductions in under-5 child mortality (7).

Reflecting this experience, WHO has identified specific criteria to guide global NCD indicator and target development (8). They include that indicators have unambiguous measurement tools, epidemiologic and public health relevance, and relate to established priorities and strategies. Targets must be supported by evidence that they are achievable, with effective and feasible evidence-based interventions available (2). Proposed indicators and targets meet these criteria variably. For example, a clearly defined set of interventions with demonstrated effectiveness at the national level exists in support of the Framework Convention on Tobacco Control, yet evidence-based nationally scalable models to challenge upward trends in global obesity are yet to be determined.

The technical process of indicator selection has been guided by a focus on the four modifiable risk factors that lay claim to the majority of NCD-related morbidity and mortality: tobacco use, unhealthy diet, physical inactivity, and harmful use of alcohol. None of these risk factors can be fully addressed through work in a single sector. For example, diet is influenced by culture, geographic location, income, education, and available foods, which in turn are influenced by city planning and infrastructure, transportation, trade, agriculture, and energy, just to name a few.

Determinants of NCDs go well beyond the traditional domains of public health. Yet the process of indicator and target setting is headed by the UN specialized health agency, WHO, with technical input largely from health experts and member state consultations dominated by ministries of health. It is thus no surprise that proposed indicators with targets seem to focus on health measures. Proposed indicators include tobacco use, raised blood pressure and serum cholesterol, physical inactivity, harmful use of alcohol, obesity, high salt and fat intake, treatment of those at risk for cardiovascular disease, and access to medications and technology. Where is the call for government multisector response and engagement from private industry and civil society? Is this a failure to capitalize upon the UN HLM, which transcended boundaries of health by engaging heads of state?

A comprehensive set of global indicators and targets specific to the multitude of relevant parties is not feasible. This would require years to negotiate and resources for monitoring well beyond those that exist. A technical solution is to select indicators proximate to NCDs and risk that will capture the aggregate of contributions from a multitude of sectors. Experience from implementing the MDGs also favors selection of indicators that capture multiple interventions (6) rather than singly focused measures. For example, an indicator that measures the prevalence of raised blood pressure, one of the leading global contributors to death, would reflect the combined impact of medical treatment for hypertension, as well as initiatives to improve diet (e.g., to reduce dietary salt), increase physical activity, and decrease alcohol intake. These actions will require contribution from the private sector, such as the food and beverage industry, agriculture, and pharmaceuticals, and from multiple government sectors, such as transit and energy, and others. Bringing these stakeholders to the table will be no easy task and is the subject of two related UN HLM mandated processes: an update of the *WHO Action Plan on the Global Strategy for the Prevention and Control of NCDs* (9), which is expected to include a road map for implementation of the commitments from the UN HLM, and a report on strengthening multisectoral action.

Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

\*Author for correspondence. E-mail: sangell@cdc.gov



### Marrying Measurability, Form, and Function

If an indicator does not include a feasible and valid mechanism of measurement, analysis of resulting data may indicate success where there was none or report failure in the face of achievement. This may support inappropriate use of resources and missed opportunities to prevent disease or death. For example, although there are some objective measures of NCD risk factors, such as direct measurement of weight or analysis of 24-hour urine collection for salt intake, measurement of many behavioral risk factors will depend on surveys where respondents self-report their actions. These data may be less reliable, as responses are influenced by culture, environment (such as rural versus urban), ethnicity, race, and gender, among other variables. The adopted indicator for mortality relies on vital registration systems, which only exist at the level of completeness needed to determine all-cause death in about two-thirds of the world's countries (2) and cannot be reliably disaggregated to estimate specific cause of death in most. To increase reliability, the global mortality indicator and target were created as an aggregate, capturing all major causes of NCD mortality, rather than a separate target for each individual cause of NCD death.

Data limitations are expected, as data collection and reporting, especially in the setting of nascent surveillance systems, require resource investment. For these reasons, careful consideration of the capacity of the tool to measure the variable of interest consistently across populations, within countries, as well as between countries over time, should be central. Additional resource investment will be needed for surveillance and vital registration systems capturing risk and cause-specific morbidity and mortality.

For many NCD risk factors, such as blood pressure and serum cholesterol, risk varies over a continuum. Within a normal range, lower is generally better, but no specific level indicates “no risk.” Rather than focusing on just those at the high end of the distribution, interventions that shift the mean risk of an entire population may result in greater reductions in events. Indeed, a large proportion of heart attacks and strokes occur in people without “raised blood pressure,” that is blood pressure below 140/90 mmHg, the level at which hypertension is defined for most. Yet, for many conditions, interventions targeting populations and high-risk individuals are conducted concomitantly (10). How should the spectrum of interventions for a specific condition and the continuum of population risk influence the design of indicator development? When a condi-



Port-au-Prince, Haiti, January 2010. A nurse takes a woman's blood pressure at a makeshift camp soon after the earthquake.

tion is common globally—for example, raised blood pressure, which affects 40% of the world's adults—the discussion may be largely academic. The impact of interventions targeting populations and high-risk individuals will both be captured by an indicator defined by mean population blood pressure or by percentage of the population with blood pressure below 140/90 mmHg.

In this case, a key function of indicators—to communicate information on the status of a health condition to the broader community—should guide the decision. Our experience suggests that, compared with a mean reduction in population risk, an indicator that reports the percent of people at risk is more easily understood by the lay community and policy- and opinion-makers and should be used as the form of indicator.

The better we are at preventing death from communicable diseases, the more likely we are to die from NCDs. The opportunity is not to eliminate NCDs, but rather to limit early disease by reducing modifiable risk factors. To translate this goal into an indicator and target, the population measured should be limited to those whose risk can be modified. For example, the approved mortality target for NCDs is a measure of premature mortality: reduce the probability of death in the population ages 30 to 70 years by 25% by 2025 (2).

### The Technical Process

Scientific consensus takes time to be achieved and is uncommon in new and evolving areas, such as NCDs. Tobacco use may be the best example of global scientific consensus with respect to risk and evidence for action, and this took decades to achieve. The technical process of developing indicators and targets provides a means to make recommendations based on the best evidence, but it cannot be a forum to reconcile all related scientific debates. Further, the direction of the technical process is

ultimately defined by member-state decisions, as part of the political process. For example, at the WHA in May 2012, indicators and targets that would require further development by WHO were specified by member-state agreement. The recent set of proposed indicators and voluntary targets, including those for obesity, fat intake, harmful use of alcohol, serum cholesterol, and the availability of essential medicines for NCDs, reflect this demand. Regional WHO meetings will discuss the proposed indicators

and targets. WHO will hold a final member-state consultation in November 2012, before final revision.

Although ultimately this process should strive to produce indicators and targets of scientific excellence, as Voltaire wrote, “the best is the enemy of good.” The best technical set of measurable, ambitious, and achievable indicators with targets is the ideal, but in the end, it is not the indicators or targets, rather the political commitment to achieve them, that is the main determinant of outcomes. Final endorsement by member-states, and interventions to prevent and control NCDs, are the real prize.

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# A Measurable Planetary Boundary for the Biosphere

Steven W. Running

Forty years ago, Meadows *et al.* published a landmark first analysis of global limits to human activity (1). Based on a primitive computer model of the Earth system, they concluded that by the early decades of the 21st century, tangible limits to key global resources would begin to emerge. A reanalysis of the original results in 2008 found that the original global resource depletion projections were remarkably accurate (2). Since then, Rockström *et al.* (3) have defined a new term—planetary boundaries—to describe nine variables of high importance to habitability of Earth, including climate change, ocean acidification, land-use change, and biodiversity loss. These metrics are compelling conceptually, but many are not easily measured globally; explicitly defining a critical boundary is even more challenging. I suggest a new planetary boundary, terrestrial net primary (plant) production (NPP), that may be as compelling conceptually, integrates many of the currently defined variables, and is supported by an existing global data set for defining boundaries.

Terrestrial plant production is the foundation of the biospheric carbon cycle. Using solar energy, water and atmospheric CO<sub>2</sub> are transformed into plant carbohydrate matter. This plant matter then sustains the global food web and becomes the source of food, fiber, and fuel for humanity. NPP integrates aspects of five of the currently defined planetary boundaries: land-use change, freshwater use, biodiversity loss, and global nitrogen and phosphorus cycles. It is also influenced directly by two others, climate change and chemical pollution.

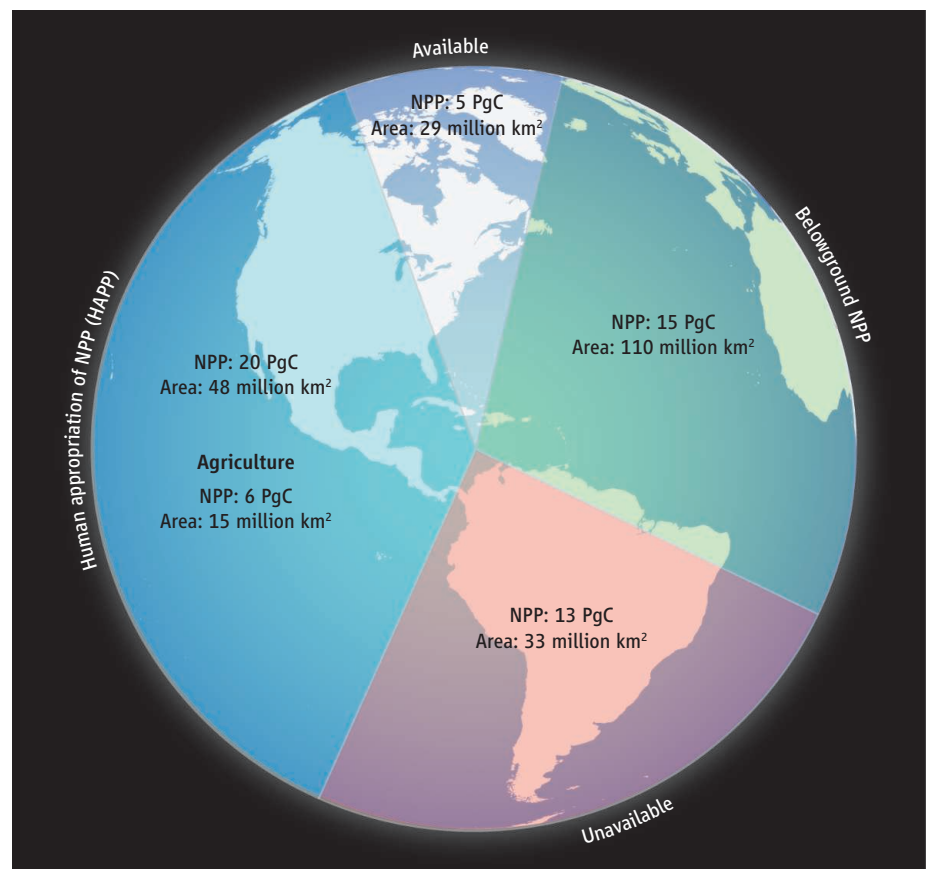
In 1986, Vitousek *et al.*, synthesizing many ground-based data sets, estimated that humans were consuming or directly co-opting 40% of biospheric production (4); they later postulated that humanity may one day consume all available NPP (5). Newer estimates that use satellite data but somewhat different assumptions agree that humans now co-opt roughly one-third of terrestrial NPP (6). Global NPP is now

derived from satellite measures of vegetated cover and density, combined with daily weather observations to analyze light, temperature, and water constraints to plant growth. In two recent papers, we presented the patterns and trends of global NPP, first from 1982 to 1999, where a small increase was observed (7), followed by an analysis with a new satellite from 2000 to 2010, where a small decrease was observed (8). However, looking at the complete data set, the most striking observation is that for more than 30 years, global NPP has stayed near 53.6 Pg per year, with only ~1 Pg of interannual variability. Thus, this key factor

Terrestrial net primary (plant) production provides a measurable boundary for human consumption of Earth's biological resources.

in the global carbon cycle seems to vary less than 2% annually.

This remarkable consistency of global NPP may be due to equally small global variability of the key drivers. The key global input of energy that drives photosynthesis, solar radiation, varies by less than 0.001% from year to year. Total global precipitation is thought to vary by only around 0.05 mm/day, or 2% each year. Thus, although there is huge regional variability within the Earth system—for example, due to droughts, floods, and heat and cold waves—the final planetary totals of energy and mass flows may average out.



**Will human consumption of primary plant production soon reach its limits?** In their original planetary boundaries diagram, Rockström *et al.* (3) identified three systems—climate change, rate of biodiversity loss, and human interference with the nitrogen cycle—that may already have exceeded their planetary boundary. However, many of these metrics are not easy to measure globally. Net primary production (NPP) integrates five of these boundaries and may be more easily measurable on a global scale. Consideration of current land use patterns and the projected rise in the human population suggest that human consumption may reach the global NPP boundary within the next few decades.

Numerical Terradynamic Simulation Group, University of Montana, Missoula, MT 59812, USA. E-mail: swr@ntsg.umd.edu



If global NPP is fixed by planetary constraints, then no substantial increase in plant growth may be possible. Hence, the obvious policy question must be whether the biosphere can support the 40% increase in global population projected for 2050 and beyond.

To determine if humanity can co-opt a higher fraction of global NPP, the previously defined planetary boundaries become relevant, starting with land use. According to the most recent estimates from global satellite data sets, humans currently appropriate 38% of global NPP, which would appear to leave 62%, or about 33 Pg, available for future consumption (see the figure) (9). However, the authors also estimate that 53% of global NPP is not harvestable. This nonharvestable part includes plant growth in root systems, preserved land (for example, in national parks that are critical for ecosystem services and biodiversity), and wilderness areas where no transportation exists for harvesting. If one subtracts this unavailable NPP, only about 5 Pg, or 10% of total global NPP, theoretically remains for additional future use by humans.

Agriculture now consumes 38% of the global land surface, with major new expansion only available in underdeveloped parts of South America and Africa (10). Land put in to agriculture often has lower production than the natural ecosystem replaced, but growth is concentrated in the components that humans value. Crop production exceeds the natural

ecosystem when augmented with irrigation and fertilizer applications. Cropland under irrigation has roughly doubled in the last 50 years, and fertilizer use has increased by 500% (10).

Many analyses now conclude that freshwater use for irrigation has already reached a planetary boundary. As some rivers are completely drained for agriculture and groundwater withdrawal limits are reached in some regions, irrigated crop area could decrease in coming decades (11). Likewise, Rockström *et al.* (3) concluded that the nitrogen and phosphorus cycles may have already exceeded planetary boundaries, as evidenced by massive river pollution and ocean anaerobic dead zones. If anything, future increases in NPP must be achieved with less, not more, irrigation and fertilizer use.

Possibly the biggest unknown in this global analysis is the future of bioenergy. If every chloroplast of the remaining 5 Pg of NPP were used for bioenergy, only 40% of current global primary energy consumption would be satisfied (9). There will be very real policy dilemmas if land previously allocated to food production is transformed to bioenergy production, raising food prices for the people who can least afford it (12).

Any analysis of global biospheric limits includes many assumptions and considerable uncertainties. Yet, global monitoring will document every parcel of land that is

converted from a natural ecosystem to cities, agriculture, or bioenergy. Every such conversion increases the fraction of NPP consumed by humanity. The question is thus not whether humans will reach the global NPP boundary but when we will do so. The projected 40% increase in human population by 2050 CE, combined with goals to substantially improve standards of living for the poorest 5 billion people on Earth, implies at least a doubling of future resource demand by 2050. As suggested 40 years ago (1), the limits to growth as measured by human consumption of NPP may well be reached in the next few decades.

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## MATERIALS SCIENCE

# Nanometer-Scale Printing

John A. Rogers

Progress in nanotechnology relies on the ability to fabricate structures with precisely defined, nanoscale dimensions. Historically, this task has been accomplished with energetic beams of electrons, ions, or photons, using sophisticated tools whose origins lie in the semiconductor industry (1). Although well suited for manufacturing of integrated circuits and related devices, such techniques are often not the best choices for exploratory research because they require expensive equipment and specialized facilities. They also tend to work well only with narrow classes of materials, and they can be prohibitively slow for use over large areas.

On page 1517 of this issue, Liao *et al.* (2) introduce a scheme that bypasses these limitations, in which rubber stamps affect nanoscale pattern transfer via molecular-scale fracture. Their technique represents a conceptual advance on a class of “soft lithographic” methods in which elastomers with fine features of relief on their surfaces deliver molecules (3, 4) or materials (5, 6) onto substrates of interest, in a process of contact printing. By providing advanced nanofabrication capabilities to researchers with limited access to complex apparatus, these simple methods have played a central role in the emergence of nanotechnology as a broad, vibrant field of study.

The work of Liao *et al.* is important in this context because it enhances the resolution of one of the most widely used soft litho-

**A method using rubber stamps and molecular-scale fracture can produce patterns with feature sizes in the nanometer range.**

graphic techniques, in which stamps made of poly(dimethylsiloxane) (PDMS) print molecules onto substrate surfaces with which they covalently react to form densely packed, monolayer films, referred to as self-assembled monolayers (SAMs) (3). This process, even when carried out by hand in an ordinary laboratory environment, can yield patterns of SAMs with features as small as a fraction of a micrometer. Two aspects, however, frustrate operation in regimes of resolution that are relevant to the frontiers of modern nanotechnology (7–9). First, the molecules that form the SAMs diffuse slightly along the surface during and after printing, thereby blurring the edges of the patterns. Second, gas-phase transport can carry molecules from the recessed, noncontacting regions of the stamp to the substrate, yielding partial monolayers

Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. E-mail: jrogers@uiuc.edu



## Nanometer-Scale Printing

John A. Rogers

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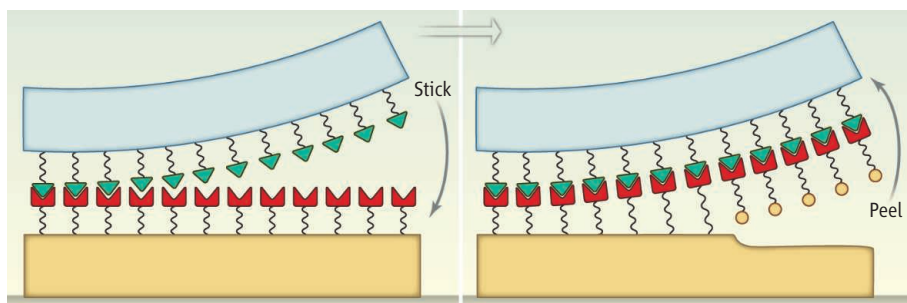
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Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. E-mail: jrogers@uiuc.edu



**Stick it and rip it.** In a new, high-resolution method for nanofabrication, Liao *et al.* show that rubber stamps (top; blue) with siloxyl groups (green) on their surfaces react covalently with hydroxyl-terminated (red) self-assembled monolayers (SAMs) on gold (bottom; gold) at points of physical contact. Peeling the stamp away initiates fracture near the surface layer of the gold, yielding SAMs with precisely patterned geometries.

in the unprinted areas. Past attempts to minimize these effects achieved some limited success through the use of molecular inks with large molecular weights (9). Liao *et al.* take a completely different approach, with even better results. Instead of using stamps to print molecules onto bare surfaces in the usual way, they exploit chemically functionalized stamps to remove molecules from preformed, unpatterned SAMs. Here, upon physical contact, covalent bonds form between the stamp and reactive groups exposed on the surface of the SAM. Peeling the stamp away “mechanically desorbs” molecules from the SAM in regions defined by contact with the stamp. SAMs patterned in this way can then serve as molecular templates for etching the underlying substrate or for guiding the deposition of other materials (2–4). The most important practical aspect of this technique, termed chemical lift-off lithography, is that its resolution in patterning SAMs exceeds that of previous soft lithographic techniques.

Optimized surface chemistries are critical to the successful operation of the process. Liao *et al.* find that Si-OH groups on PDMS and hydroxyl-terminated SAMs on gold rapidly and covalently react to form strong Si-O-SAM linkages that remain intact as the stamp is removed. Here, mechanical fracture occurs within a near-surface region of the gold, such that both the SAM and, roughly, a monolayer of gold atoms peel off of the substrate (see the figure). These steps of contact-induced chemistry followed by nanoscale fracture can occur in minutes, over areas limited only by the sizes of the stamp and substrate, and with efficiencies of SAM removal that approach ~80%. Furthermore, the edges of the patterns can be extremely sharp and well defined, with roughness at the level of only a few nanometers. As a result, patterns with dimensions in the nanometer regime are possible. Liao *et al.* demonstrate 40-nm features, apparently limited only by the sizes of the relief features on the stamps.

The capabilities demonstrated by Liao *et al.*, especially with such an extremely simple printing method, offer powerful modes of use in research, particularly when overlay registration is not required. Further development of the technique to eliminate such constraints will require engineering innovation. Fundamental extension of the resolution

will demand improved understanding of the underlying mechanisms and, in particular, the relative roles of the molecular chemistry of the SAMs, the materials science of the substrate and stamp, and the physics of nanoscale fracture. Such topics represent appealing opportunities for interdisciplinary work, with strong potential for impact in nanoscience and nanotechnology alike.

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#### STRUCTURAL BIOLOGY

## Versatility from Protein Disorder

M. Madan Babu<sup>1</sup>, Richard W. Kriwacki<sup>2</sup>, Rohit V. Pappu<sup>3</sup>

Synergy between disordered regions and structured domains increases the functional versatility of proteins and their interaction networks.

Many protein functions can be attributed to segments (domains) that fold independently and adopt specific three-dimensional structures (1). Intrinsically disordered regions (2, 3) are unstructured segments whose amino acid compositions prevent autonomous folding. Some eukaryotic proteins are either fully disordered (intrinsically disordered proteins) or structured, but most have both types of regions (see the figure). The notion that disordered regions are largely passive is being actively challenged by the idea that they perform diverse functions, and that synergy between structured and disordered regions expands the functional repertoires of proteins.

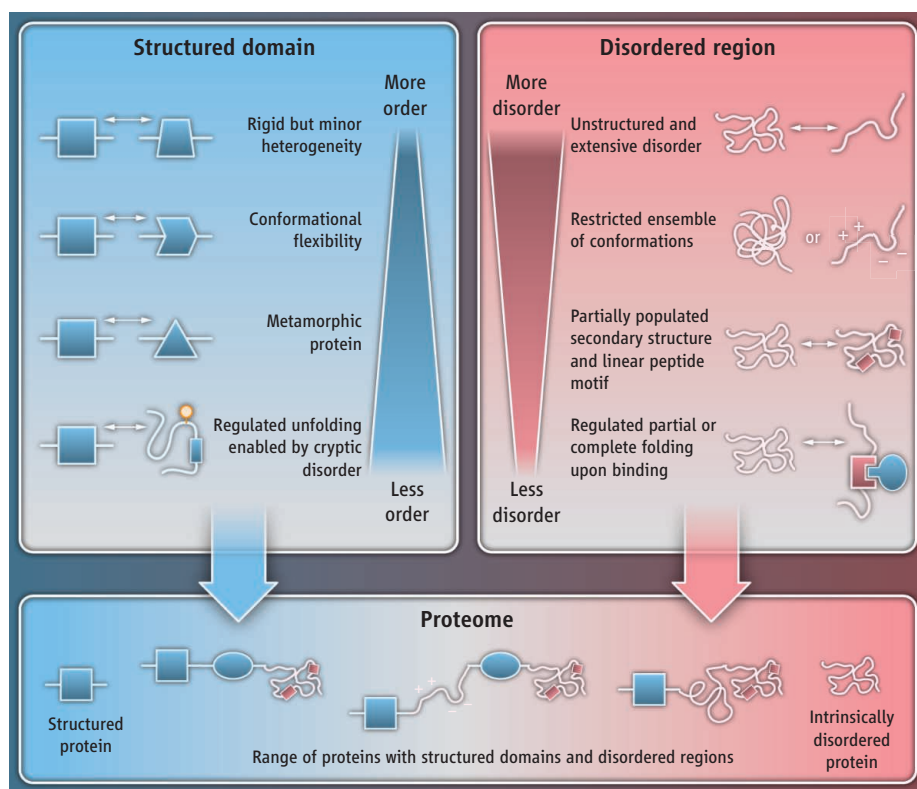
Understanding how disordered regions mediate function requires accurate physical descriptors of sequence-disorder relation-

ships. Recent studies based on a combination of polymer theory, computer simulations, and biophysical experiments have revealed some coarse-grain conformational properties of disordered regions. Sequences enriched in polar amino acids and deficient in hydrophobic residues form compact, globular conformations (4, 5). As the net charge per residue within disordered regions increases, they undergo continuous transitions to loosely packed ellipsoidal random coils (6, 7).

The growing list of features attributed to disordered regions suggests that they act as molecular rheostats to support a continuum of conformational states and transitions. These features enable disordered regions to mediate highly specific interactions with multiple binding partners (2, 3). Conformational fluctuations of these regions can control the exposure of short linear motifs (8) that interact with protein domains, thereby regulating protein interactions. Posttranslational modifications within or near these linear motifs may modulate conformation and affinities, thus increasing the functional capabilities of disordered regions. Examples include the tails

<sup>1</sup>MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK. <sup>2</sup>Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA. <sup>3</sup>Department of Biomedical Engineering, Washington University of St. Louis, St. Louis, MO 63130, USA. E-mail: madanm@mrc-lmb.cam.ac.uk; richard.kriwacki@stjude.org; pappu@wustl.edu





**Determinants of protein function.** Structured domains and disordered regions are fundamental units of protein function. Most proteins in eukaryotic proteomes contain both types of regions. The bar thickness shown for each type of region indicates a continuum and extent of conformational heterogeneity.

of histone proteins, several receptor kinases, and proteins that control the cell division cycle (2, 3, 9). A disordered region can also display multiple motifs, giving rise to multivalent interactions that drive the formation of micrometer-sized assemblies as recently reported for actin regulatory proteins (10). These assemblies may afford higher-order spatial organization and increase the local concentrations of proteins, and thereby regulate diverse cellular processes. Further, it was recently observed that the number and density of linear motifs within disordered regions can be regulated posttranscriptionally by alternative splicing of messenger RNA. Tissue-specific splicing events appear to alter the disordered regions that harbor binding motifs while leaving the structured regions intact (11). This can lead to rewiring of molecular interaction networks and new functional consequences. For example, a nearby structured region, such as a kinase domain, may become capable of acting on (phosphorylating) different proteins (new substrates) that bind to the spliced, disordered segment containing the linear motif. Given the critical roles mediated by such regions, the abundance of proteins with disordered regions is highly regulated to prevent nonfunctional promiscuous interactions that can cause disease (12).

Upon binding to an interaction partner, disordered regions can either undergo disorder-to-order transitions or preserve their disordered state by forming “fuzzy complexes” (13, 14). Specific regions within structured domains (or folded regions of a bound disordered region) undergo regulated unfolding. This form of cryptic disorder (15) is an example of emergent behavior as it can generate new disordered states by coupling a binding event or posttranslational modification of structured domains to an order-disorder transition. Although current computational methods can readily identify disordered regions, uncovering regions of cryptic disorder encoded within structured domains remains a challenge. In addition, disordered regions also have regulatory roles that include an ability to induce local unfolding within adjacent structured domains and facilitate allosteric communication between structured domains.

How do disordered regions evolve? Because they do not require a defined structure for their function, disordered regions may be more tolerant of mutations than structured domains. This might confer an advantage if a new motif emerges through convergent evolution. Although evolutionarily conserved motifs can be identified through sequence analysis, the discovery of new motifs that are

not conserved but are still functional represents a challenge for the future. If sequences of disordered regions change rapidly, do such mutations increase the risk of disease? Analysis of diverse cancer-associated mutations shows a dominant clustering within structured regions of proteins, suggesting that disordered regions provide robustness to mutation-induced effects on phenotype (16). Continued studies of sequence variations in normal and diseased cells are necessary to fully understand the contributions of disordered regions to fitness and disease.

Although in vitro experiments and molecular simulations provide important insights, disordered regions need to be studied in biologically relevant contexts to understand how complex functions emerge through the synergy between structured domains and disordered regions. Controlling protein function by modulating the degree of disorder through sequence design should reveal how natural variations in disordered regions affect the emergence of new phenotypes. These challenging issues are inspiring the development of new interdisciplinary approaches, from studies of single molecules and protein ensembles in cells, to deciphering the behavior of disordered proteins within entire biological systems. Complementing computational approaches, functional studies, and systems-level analyses of disorder with biophysical investigations will yield important insights regarding sequence-disorder-function relationships.

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## Thermally Responsive Pulsating Nanotubules

Wei Zhang and Takuzo Aida

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## CHEMISTRY

# Thermally Responsive Pulsating Nanotubes

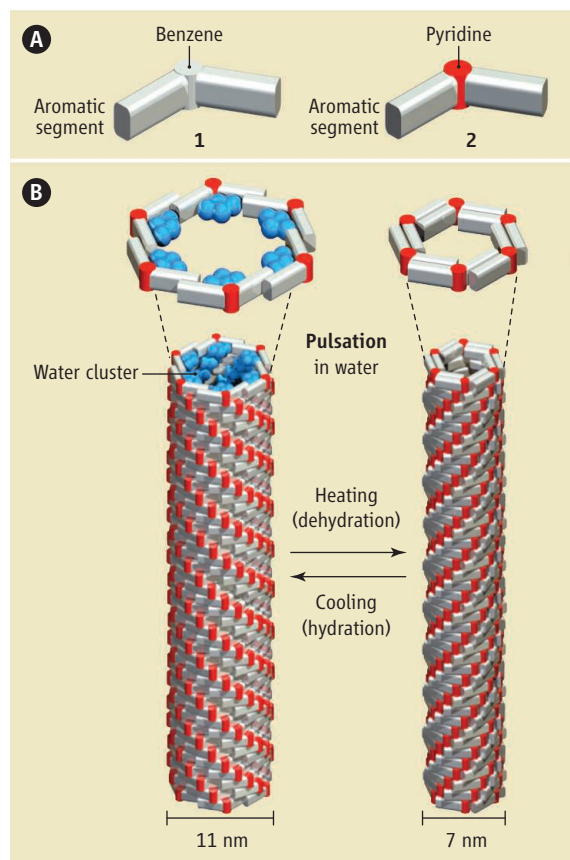
Wei Zhang<sup>1</sup> and Takuzo Aida<sup>1,2</sup>

Thousands of years ago, Chinese medical doctors started to diagnose illness simply by feeling a pulse at a wrist with two fingers, knowing that the periodicity of the beating heart could be diagnostic (1). One of the ultimate scientific challenges would be to develop artificial analogs of cardiac muscle cell that pulsate autonomously. One approach has been to use noncovalent molecular assemblies (2) that pulsate in response to external stimuli. Essential issues to consider are what mechanism can be used to drive pulsation and how to visualize the resulting motion. Previous examples have featured “pulsating vesicles” that responded to pH and light as stimuli (3, 4). On page 1521 of this issue, Huang *et al.* (5) report a thermoresponsive nanotube that undergoes shrinkage and expansion upon heating and cooling, respectively, in water (5). By making use of this thermoinduced pulsation, the nanotube can push out guest molecules such as C<sub>60</sub> from its hydrophobic interior.

The authors took advantage of the thermoresponsive hydration and dehydration of pyridine (through hydrogen bonding to its nitrogen atom) (6) and oligo(oxyethylene) in water (7). Ionic and certain neutral polymers are water soluble and are hydrated by ion-dipole and dipole-dipole interactions, respectively. Because these interactions are rather weak and also require proper orientation of water molecules along the hydrated polymer backbone (which leads to an entropic penalty and a free-energy barrier to the process), such water-soluble polymers may, upon heating, be dehydrated and undergo aggregation or shrinkage in water (7). This trend is different from that of polymers in organic solvents, which are more soluble at higher temperatures.

Huang *et al.* used a particular design strategy for making use of thermoresponsive hydration and dehydration. As a prototype of this strategy, the authors synthesized a bent amphiphilic aromatic molecule (1) with an internal angle of 120°, which carries at its apex

The nanotube formed from stacked macrocyclic units undergoes thermally induced swelling and unswelling and mimics the action of a pump.



**Swell results.** (A) Schematic structures of amphiphilic bent-shaped molecules with benzene (1) and pyridine (2) cores synthesized by Huang *et al.*; the chiral dendron unit at their apex is omitted for clarity. (B) Schematic representations are shown of the expanded and contracted states of a helically chiral nanotube formed by self-assembly of 2. Note the change in helicity. Blue moieties are water clusters, possibly formed by a hydrogen-bonding interaction of the pyridyl group with water, and are supposed to generate and lock the expanded state.

a water-soluble oligoether dendron. In water, a noncovalent macrocycle containing six molecules of 1 forms and then stacks into a tubular structure with a hydrophobic interior and a hydrophilic exterior bearing regular branched polymer units called dendrons. The macrocyclic component of the nanotube forms noncovalently through weak interactions and can change its diameter without morphological disruption by simply sliding the stacked aromatic segments with one another.

The authors noted that the aromatic segments of 1 seem to stack regularly in water, as

they formed *H*-type aggregates (one-dimensional arrays of aromatic molecules with a face-to-face arrangement). They recognized that they needed a way to slide the aromatic segments to expand the nanotube and lock the resulting expanded state in place. They replaced the central benzene core with pyridine (2), because pyridine is known to form a water cluster through a hydrogen-bonding interaction (see the figure, panel A) (6). They envisioned that such a water cluster, if formed within the nanotube, might lock its expanded state. Upon heating, its thermal breakdown would allow sliding motion of the aromatic segments and cause the nanotube to shrink.

This small alteration in the molecular structure did indeed bestow the desired thermal response on the nanotube. Upon heating to 60°C, the nanotube changes its inner and outer diameters from 4 and 11 nm, respectively, to 3 and 7 nm, thereby furnishing a 47% reduction in its cross-sectional area (see the figure, panel B). The entire process of pulsation is fully reversible. The authors also found that coassembly of 2 and a fullerene such as C<sub>60</sub> formed a peapod type of nanotube containing C<sub>60</sub> in its hydrophobic interior (8).

Upon heating, the shrinking nanotube pushed out some of its fullerene guests.

The nanotube from 2 is optically active because of a helical superstructure with a preferred handedness imparted by the chiral dendron unit. Namely, depending on the absolute stereochemistry of the dendron unit, the hexameric macrocycle of 2 stacks up either in a clockwise or counterclockwise manner to form a one-handed helical chirality in the resulting nanotube. Surprisingly, the helical sense of the nanotube switches from one to the other in the pulsation event.

<sup>1</sup>Riken Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. E-mail: zhangwei@riken.jp

<sup>2</sup>Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan. E-mail: aida@macro.t.u-tokyo.ac.jp

Huang *et al.* successfully visualized this helical inversion by atomic force microscopy. Hence, not only a small aromatic sliding motion within the constituent macrocycles but also reorganization of the stacking architecture of the macrocycles accompanies the pulsation. Such a large structural reorganization is most likely caused by dehydration of the water-soluble dendron unit upon heating (7).

The work highlighted herein is indeed

stunning and seminal. In contrast with precedent “pulsating vesicles,” one-dimensional hollow structures, if capable of pulsating, could generate an anisotropic mechanical force (flow of fluids), which may transport cargoes unidirectionally. Thermal energy is isotropic and dissipative. Upon further elaboration, thermo-responsive “pulsating nanotubes,” just like cylinders in engines, may be able to convert thermal energy into an anisotropic motion.

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## CANCER

# Emerging Anatomy of the BAP1 Tumor Suppressor System

Anne E. White and J. Wade Harper

The genetic drivers of cancer are incredibly complex; they include frequent mutations in a small number of tumor suppressors and oncogenes, as well as a large landscape of rare genetic variants that may promote tumorigenesis in a lineage-dependent manner. A recent flurry of papers has demonstrated prevalent somatic and germline mutations in a novel tumor suppressor, the deubiquitinating enzyme BAP1, in a variety of tumor types, including some with metastatic potential (1–5). Through analysis of mice lacking BAP1 and human tumors, Dey *et al.* now identify BAP1 as a tumor suppressor in myelodysplastic syndrome (MDS), a bone marrow and blood cell cancer syndrome that often evolves into highly aggressive acute myelogenous leukemia (AML), as reported on page 1541 of this issue (6). Functional analysis of the BAP1 protein interaction network in vivo, together with target gene identification, revealed a transcription complex in which ubiquitin removal from regulatory components by BAP1 likely specifies the repertoire of genes underlying tumor suppression.

BAP1 was discovered more than a decade ago as a protein that can associate with the tumor suppressor BRCA1 (7). BAP1 overexpression was shown to suppress tumor cell expansion in mouse xenografts, consistent with a tumor suppressor role, but surprisingly this effect did not require BRCA1 (8). The identification of BAP1 as a bona fide tumor suppressor came through exome sequencing of sporadic tumors and genetic analysis

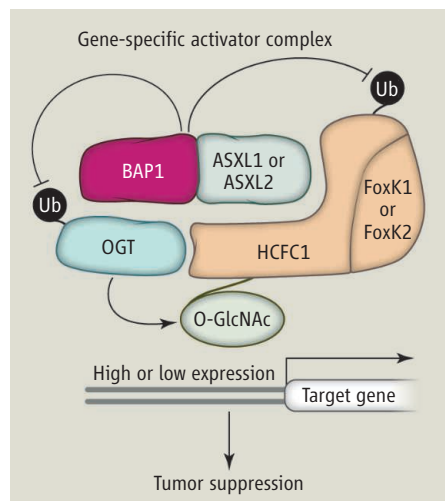
of familial cancer predisposition syndromes (1–5). Deletions and/or point mutations in the *BAP1* gene are present in melanoma, mesothelioma, lung adenocarcinoma, meningioma, and renal cell carcinoma. In some cases, a high percentage of patients carry *BAP1* mutations; for example, ~80% of metastasizing uveal melanomas have sporadic or germline *BAP1* mutations (1).

Dey *et al.* found that whereas deletion of *BAP1* during embryogenesis is lethal, *BAP1*

Analysis of the tumor suppressor BAP1 reveals how its associated transcriptional complex may suppress tumors of different lineages.

loss in the adult mouse hematopoietic lineage leads to a myeloproliferative/myelodysplastic disorder with features of human chronic myelomonocytic leukemia (CMML). Phenotypes include elevated levels of blood cell precursors (myeloid cells) in the spleen, abnormal progenitor red blood cells (myelodysplasia), and low platelet count. CMML-like disease is due to BAP1 loss specifically in the hematopoietic compartment, as demonstrated by bone marrow transplantation experiments. Consistent with a role in MDS in humans, Dey *et al.* identified a heterozygous nonsense mutation in the hydrolase domain of BAP1 in 1 of 32 tumors from de novo MDS patients. Interestingly, mild but progressive hematological defects mirroring those found in MDS patients were also seen in hematopoietic lineage BAP1<sup>+/-</sup> mice, suggesting that BAP1 may be a haploinsufficient tumor suppressor in some contexts.

Protein ubiquitination is a reversible post-translational modification regulating many cellular processes. BAP1 suppresses tumorigenesis through its deubiquitinase activity (1–5, 8), but in what contexts and through what mechanisms does BAP1 act? Initial insights came from analysis of BAP1-associated proteins in tissue culture cells, wherein BAP1 was found to associate with an array of proteins related to chromatin and transcription, albeit not BRCA1 (9–12). Although the structural architecture and stoichiometry of BAP1 complexes have not been elucidated, the simplest model (see the figure) is one in which BAP1 forms a modular complex with (i) the HCFC1 transcriptional scaffolding subunit (host cell factor-1, also called HCF-1) previously linked to transcriptional control of cell cycle regula-



**Modes of tumor suppression.** Interaction of BAP1-ASXL1 with HCFC1-FOXK1 allows deubiquitination and thereby stabilization of OGT, HCFC1, and potentially other unknown targets (not shown), allowing O-GlcNAc modification of HCFC1 by OGT to regulate transcription. The BAP1-ASXL1-HCFC1-OGT complex localizes on many gene promoters, possibly through factors such as FOXK1, and can likely function as either a gene-specific activator or repressor complex on distinct genes to control tumor suppression in certain cell lineages.

Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA. E-mail: wade\_harper@hms.harvard.edu



tory genes [reviewed in (13)]; (ii) an O-linked *N*-acetylglucosamine (O-GlcNAc) transferase subunit (OGT), which modifies HCFC1 (14); (iii) one of two related BAP1-activating subunits (ASXL1 or ASXL2) (15); and (iv) one of two sequence-specific DNA binding proteins (FOXK1 and FOXK2) that may provide target gene specificity (9–12).

The BAP1 complex is remarkable in two respects. First, ASXL1 is mutated in CMML (6). Assuming that ASXL1 and BAP1 mutations are mutually exclusive, this suggests that BAP1-ASXL1 is central to suppression of myelodysplastic/CMML disorders, and also implies that ASXL1 may be mutated in other tumor types with frequent BAP1 loss. Second, orthologs of BAP1 and ASXL1 in *Drosophila*—Calypso and Asx—are components of the polycomb (PcG) repressor complex that controls *hox* gene expression presumably through deubiquitinating histones (15). Calypso lacks the motif that interacts with HCFC1, so at face value, the major BAP1-Calypso complexes in humans and *Drosophila* are different in form. Moreover, BAP1 deficiency did not result in changes in HOX transcripts in hematopoietic cells (6). Also, the HCFC1 complexes associated with BAP1 appear to be distinct from the HCFC1-methyltransferase complexes previously linked with proliferative control (13).

To probe BAP1 function in vivo, Dey *et al.* used quantitative proteomics to identify BAP1 complexes from the brains and spleens of mice, identifying BAP1-ASXL1-HCFC1-OGT-FOXK1, as well as interactors yet to be validated. But which targets are important for the tumor-suppressive function of BAP1? HCFC1 is known to be ubiquitinated, which is reversed by BAP1 (see the figure), raising the possibility that HCFC1 itself is a critical target (10–12). Consistent with this, BAP1 mutants that cannot interact with HCFC1 fail to fully suppress cell growth in vitro (5, 11). Yet the precise mechanisms of regulation are unclear and may be context-dependent, as HCFC1 levels are unaltered when BAP1 is introduced into BAP1-deficient renal cancer cells (5), whereas HCFC1 levels are dramatically reduced in extracts from BAP1<sup>−/−</sup> splenocytes (6). Consistent with the potential for additional BAP1 substrates in the complex, Dey *et al.* found that OGT was more rapidly degraded in the absence of BAP1 and that BAP1-ASXL1 could remove ubiquitin from OGT in vitro (see the figure). Because OGT serves as a positive regulator of HCFC1 (14), BAP1-ASXL1 may function to stabilize OGT and thereby keep HCFC1 active.

Because BAP1-ASXL1 is expected to regulate HCFC1-dependent transcription, Dey *et*

*al.* used chromatin immunoprecipitation and DNA sequencing (ChIP-seq) from macrophages to identify ~6000 candidate binding sites of the BAP1-containing transcriptional complex on ~5700 genes, the majority of which are also associated with HCFC1 and OGT. Of these, 32 were down-regulated and 18 were up-regulated in BAP1<sup>−/−</sup> cells, including several immune system genes, painting a complex picture of the BAP1-associated transcriptional network.

These studies provide a molecular framework for further elucidating the mechanisms by which BAP1 suppresses tumorigenesis. A key unanswered question concerns how the complex array of BAP1- and HCFC1-dependent transcripts creates a regulatory environment consistent with tumor suppression (see the figure). Target genes will likely be lineage-specific, but in the case of the hematopoietic system, the interleukin 7 receptor has emerged as a candidate, as it is required for cell survival (6) and its levels are reduced in MDS. The answer to this question likely requires an analysis of how the BAP1 com-

plex is recruited to specific promoters—for example, through associated DNA binding factors FOXK1 and FOXK2. Moreover, a clearer description of the identity and fate of BAP1 deubiquitination targets is necessary if we are to fully understand the anatomy of this important tumor suppressor complex.

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## SOCIOLOGY

# Moving and the Neighborhood Glass Ceiling

Robert J. Sampson

Moving to a lower-poverty neighborhood can have a lasting effect on individual well-being.

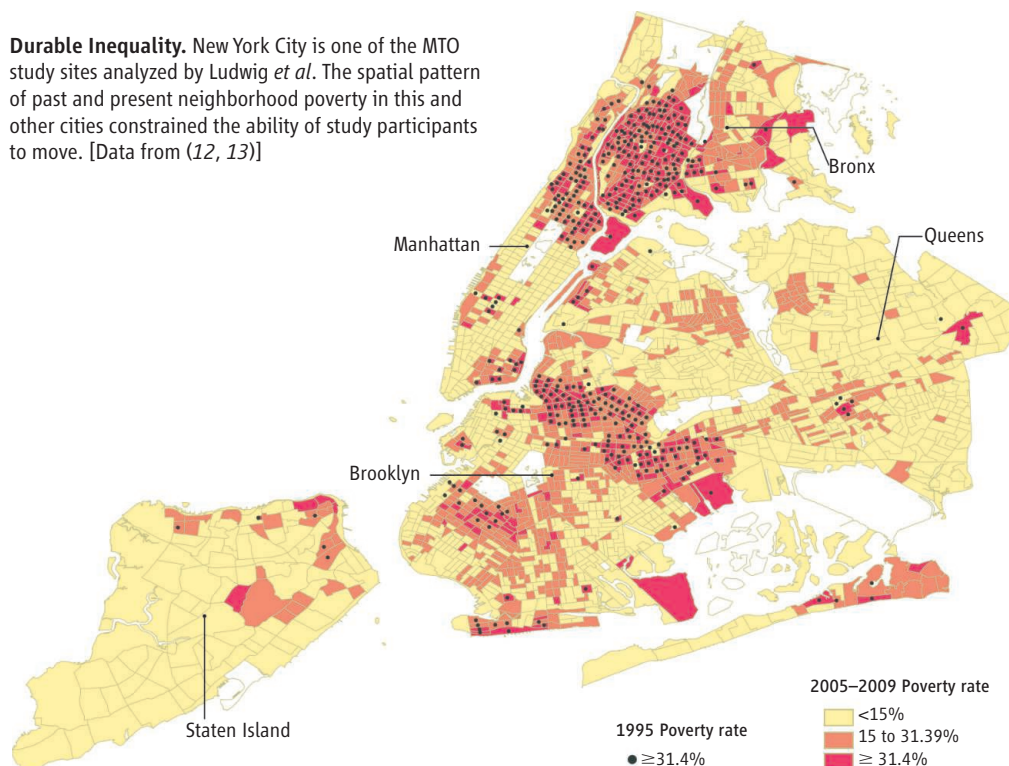
Twenty-five years ago, William J. Wilson drew widespread attention to increases in the concentration of poverty in the United States and the diminished life chances of “the truly disadvantaged” (1). The hypothesis that growing up in a severely deprived neighborhood threatens well-being is rooted in a long scientific tradition. From Victorian London to present-day America, research has shown links between neighborhood poverty and outcomes such as crime, economic dependency, poor physical health, teenage pregnancy, and school dropout (2). But could the characteristics of individuals living in deprived neighborhoods explain these observed associations rather than the characteristics of the neighborhood itself (3)? On page 1505 of this issue, Ludwig *et al.* (4) address this question by analyzing the

results of a policy project that supported neighborhood relocation.

In the mid-1990s, the Moving to Opportunity (MTO) project by the U.S. Department of Housing and Urban Development randomly assigned rent-subsidized housing vouchers to over 4500 low-income and mostly female-headed families. At the time, the families resided in public housing in extremely poor neighborhoods of New York, Los Angeles, Chicago, Boston, and Baltimore. Based on their analysis of follow-up data 10 to 15 years later, Ludwig *et al.* report that averaged across the study period, voucher recipients lived in neighborhoods where 31.4% of residents were living in poverty. For comparison, the control group lived in neighborhoods that were 39.6% poor. Although the neighborhoods of both groups were more than double the national average of 15% poor, voucher recipients had better mental health and a higher subjective well-being (happiness) than the control group at the end of the study. By contrast, there were no effects on economic self-sufficiency (such

Department of Sociology and Radcliffe Institute for Advanced Study, Harvard University, Cambridge, MA 02138, USA. E-mail: rsampson@wjh.harvard.edu

**Durable Inequality.** New York City is one of the MTO study sites analyzed by Ludwig *et al.* The spatial pattern of past and present neighborhood poverty in this and other cities constrained the ability of study participants to move. [Data from (12, 13)]



as welfare receipt and employment), and the effect on overall physical health approached but did not reach significance.

MTO's experimental design effectively eliminates individual characteristics, or "selection bias," as a rival explanation of results. Furthermore, Ludwig *et al.*'s study moves beyond the narrow confines of economic outcomes to include both physical and mental well-being. A related MTO study shows neighborhood effects on obesity and diabetes (5).

The authors also examine the long-term consequences of moving out of neighborhood deprivation. Neighborhood effects depend not only on where individuals live today, but on where they lived in the past and where their parents lived (6–8). Because the average age of MTO participants was 30 at the start of the study, Ludwig *et al.* can only estimate the conditional effect of neighborhood relocation among individuals who grew up in poor neighborhoods and lived there for years. Given this restriction, it is notable that subsequent moves to lower-poverty neighborhoods can make a lasting difference in core aspects of well-being.

Although MTO may have targeted "anti-poverty" or economic outcomes, it was more successful in changing cognitive and physical aspects of individual well-being. When residents were originally asked about reasons for moving, getting away from gangs and crime was paramount. Neighborhood

poverty is correlated with high rates of violence and other dimensions of social distress (2), and Ludwig *et al.* show significant improvement for voucher recipients in the perceived safety and social control of the neighborhood. MTO thus impacted what the residents cared most about, rather than what policy-makers deemed most important.

However, the concentration of poverty is a stubborn problem. Experimental interventions face formidable obstacles when individuals are the unit of intervention in cities marked by persistent neighborhood inequality. Only about half of the eligible participants used the voucher, in part because of difficulties in moving and in securing apartments in lower-poverty neighborhoods. Many voucher users moved to economically declining neighborhoods or eventually back to higher-poverty neighborhoods (9). By the end of the study, only 3% in neighborhood poverty separated the experimental and control groups.

There is also a strong connection between racial segregation and neighborhood poverty (10). African Americans are much more likely than whites to be exposed to severe neighborhood disadvantage (6, 7). Ludwig *et al.* found that reductions in poverty were more important than reductions in racial segregation for improving well-being, but MTO induced only minimal change in a highly segregated environment: Neighborhoods of voucher recipients averaged 83% minority, compared to 88% for controls. The

shift was thus of degree not kind.

The larger structure of inequality becomes apparent when considering New York (see the figure). Neighborhoods above 31% poor—the MTO experimental group average—are relatively rare and are embedded in clusters with a legacy of similarly high poverty. American cities in general are segmented by income and race, a persistent pattern over decades (2). Despite housing assistance, MTO participants thus faced a persistent hierarchy of neighborhoods that highly constrained individual choice. Perhaps any voucher effects are surprising.

The social mechanisms that explain the neighborhood effects seen in the MTO study are unknown, given that neighborhood poverty is correlated with many other characteristics. Theory and observational research are needed to better explain why neighborhoods matter. Research is also needed across a broader range of neighborhood environments and

outcomes, including neighborhood effects on children, intergenerational processes, and residential selection. Durable neighborhood inequality sharply influences individual housing choice and thus neighborhood-level change, but it remains unclear whether people-based or place-based interventions will be more effective in confronting persistent spatial divisions by race and class. With income segregation increasing and racial segregation remaining very high (11), the future well-being of the truly disadvantaged is a distinctive policy challenge.

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## INTRODUCTION

# It Takes More Than an Apple a Day

AS MODELS FOR PREVENTION RESEARCH, INFECTIOUS DISEASES HAVE SET A HIGH standard. Thanks to the development and widespread distribution of vaccines and antimicrobial drugs, we now live in a world free of smallpox, nearly free of polio, and with declining rates of malaria and AIDS. These victories (some still partial) have resulted in people living longer and acute infectious diseases being superseded in many countries as a public health priority by long-term noncommunicable diseases. Heart disease, metabolic disease, cancer, and respiratory disease together account for 60% of all deaths worldwide and 80% of deaths in low- and middle-income countries. Global projections for dementia are particularly alarming: By the year 2050, the disorder may affect more than 100 million people.

Logic dictates that preventing these diseases is a better approach than treating people after they have become ill. In many cases, the knowledge and tools needed for prevention appear to be in place. A number of these killer diseases share risk factors that can be modified by lifestyle changes—for example, by eliminating tobacco use, eating less processed food, and increasing physical activity. For certain cancers, screening tests are available that allow detection of the disease at an early stage. So why is prevention of these diseases so difficult when it seems like such a good idea on paper?

This special section of *Science* highlights many of the challenges facing researchers in noncommunicable disease prevention, a field characterized by impassioned debates on issues as fundamental as whether the benefits of cancer screening outweigh the risks, and which forms of prevention are the most cost-effective. The need for carefully designed clinical trials is a common theme in discussions of potential chemopreventive agents—among them aspirin, vitamin D, vaccines against chronic diseases, and  $\beta$ -amyloid-lowering drugs. The preventive strategies most likely to succeed on a population-wide scale are described, as are the best ways to integrate these efforts with infectious disease prevention, and the far-reaching effects (some adverse) that disease prevention efforts could have on a country's economy. And even as medical researchers seek new prevention drugs and strategies, we are reminded that lifelong health requires proper nutrition, especially during the first 1000 days of life, and that effective prevention will require an understanding of why people engage in health-harming behaviors.

One year ago this month, the United Nations convened a conference focused on the global reach of noncommunicable diseases, and it has since set a goal: to reduce the probability of premature mortality from these diseases by 25% by the year 2025. Although the specifics are still a work in progress, preventive strategies will probably play an important role. As if prevention researchers didn't already face enough challenges, they now find themselves working on a deadline.

— CAROLINE ASH, PAULA KIBERSTIS, ELIOT MARSHALL, JOHN TRAVIS

## Disease Prevention

### CONTENTS

#### News

- 1468 Task Force's Prevention Advice Proves Hard to Swallow  
Public Health Measures in Disease Prevention
- 1471 Will an Aspirin a Day Keep Cancer Away?  
Wondering How the Wonder Drug Works
- 1473 Tackling America's Eating Habits, One Store at a Time
- 1476 Uncertain Verdict as Vitamin D Goes On Trial
- 1479 Chronic Disease Vaccines Need Shot in the Arm

#### Review

- 1482 Can Noncommunicable Diseases Be Prevented? Lessons from Studies of Populations and Individuals  
*M. Ezzati and E. Riboli*

#### Perspectives

- 1488 Preventing Alzheimer's Disease  
*D. J. Selkoe*
- 1492 Changing Human Behavior to Prevent Disease: The Importance of Targeting Automatic Processes  
*T. M. Marteau et al.*
- 1495 Fetal and Early Childhood Undernutrition, Mortality, and Lifelong Health  
*C. K. Lutter and R. Lutter*
- 1499 Double Burden of Noncommunicable and Infectious Diseases in Developing Countries  
*I. C. Bygbjerg*
- 1501 Why a Macroeconomic Perspective Is Critical to the Prevention of Noncommunicable Disease  
*R. Smith*

See also Editorial p. 1433; Policy Forum p. 1456; Science Careers; Science Podcast; and videos at [www.sciencemag.org/special/prevention](http://www.sciencemag.org/special/prevention).

# Science

# Task Force's Prevention Advice Proves Hard to Swallow

The current chair of the U.S. Preventive Services Task Force defends the panel's controversial decisions on issues such as cancer screening

**VIRGINIA MOYER, A PEDIATRICIAN WITH A** keen interest in health screening, remembers what it was like to be on the U.S. Preventive Services Task Force (USPSTF) before it became notorious—back when “we felt like we were toiling in obscurity.” Created by the Department of Health and Human Services (HHS) 3 decades ago, the panel examines clinical data and decides whether methods of preventing disease—many of them already in use—are backed by evidence that they're worth using. Moyer's USPSTF term began in 2003 and except for a 2-year break, she has served ever since, becoming its chair in 2011. It was in 2009 that she and the rest of the task force shed their relative anonymity when they addressed a question many had thought settled: Should healthy women in their 40s get an annual mammogram?

The panel's explosive answer: No, because giving such advice would do more harm than good. It would lead to a huge number of false alarms and biopsies, the 16-member task force found, but would do little to reduce cancer deaths.

That's not how the panel worded its conclusion, however. Using carefully honed metrics, it gave a “C” grade (meaning weak) to evidence for benefits of mammography in this age group, a topic the task force had studied in various ways for years. After failing to agree on a first vote, USPSTF had commissioned new modeling studies to



**Questioner.** Pediatrician Virginia Moyer likes to ask, “Why are we doing this?”

clarify risks and benefits. Then the majority voted “against” a proposed recommendation that 40-year-olds get annual mammograms.

Turmoil ensued. Congressional committees grilled USPSTF members—nearly all of whom were physicians or nurses, and many of whom had Ph.D.s. Critics hastened to point out that none were cancer specialists. Advocates of mammography com-

plained that a nerdy committee was trying to cut medical benefits. Radiologists claimed that, maybe because panel members didn't work directly with cancer patients, they undervalued the lifesaving power of mammography. Kathleen Sebelius, the HHS secretary, went on television to urge calm, arguably undercutting the panel by asking women to “do what you've always done: ... Talk to your doctor” (*Science*, 19 February 2010, p. 936).

The noisy response was partly the result of bad timing, says Moyer, a professor at Baylor College of Medicine in Houston, Texas. The task force issued its finding in the fall of 2009 when debate over the Administration's health care reform was raging and congressional election campaigns were heating up. It was a “perfect storm,” agrees epidemiologist J. Michael McGinnis, an advocate of preventive health care and senior scholar at the Institute of Medicine in Washington, D.C.

USPSTF touched off another furor in May 2012 when it voted to reject a widely used method of screening for prostate cancer: the blood test based on prostate-specific antigen (PSA). In this case, USPSTF found strong negative evidence and concluded that routine screening of healthy men should stop. Urologists were outraged; since then, their professional associations have urged Congress to overhaul USPSTF. They want medical specialists like themselves seated on future USPSTF panels.

McGinnis says he expected some storms both within and outside government when, as a top HHS official, he created USPSTF in 1984. That's why HHS set it up as an independent body: “We wanted them to be totally unfettered by anything but the evidence in the way they went about their business.” But the nature of controversy has

## Public Health Measures in Disease Prevention

**Vaccines for infectious pathogens, often mandatory, are a widespread method of preventing diseases in a population, but organizations and countries have also tackled noncommunicable diseases with a variety of public health measures. Here are some notable examples.**

**C-ING OFF SCURVY:** Scurvy, which was recognized as far back as Hippocrates, results from a deficiency of vitamin C and prevents the formation of collagen. Scurvy was once a common problem for those on long marine voyages, killing more than a million sailors according to some estimates, but evidence slowly built that eating citrus fruits and other foods rich in vitamin C could prevent it. Ultimately, Britain's 1867 Merchant Shipping Act would mandate that sailors in the country's Royal Navy or Merchant Navy receive a daily ration of lime or lemon juice to prevent the condition.



**PASS THE SALT:** The mineral iodine plays a crucial role in the development of the body and the brain; a deficiency can lead to mental retardation, cretinism, and thyroid problems. Switzerland was the first to introduce iodized salt, in 1922, as a way to help prevent these conditions, and the United States and many other countries would soon adopt the practice.



changed since the 1980s. When USPSTF began, McGinnis says, he imagined that it would highlight the benefits of preventive services, “to show that the evidence base for prevention was even stronger than for many treatment interventions.” At the time, clinical medicine “was overwhelmingly focused on the treatment side,” McGinnis says.

McGinnis, who started in the Carter Administration and also served under presidents Ronald Reagan and Bill Clinton, agrees with Moyer that the 2009 storm over mammography was partly about politics. But he says that experts were at odds, and that unlucky timing and “poor communication” of findings contributed to USPSTF’s woes.

Ned Calonge, the physician-epidemiologist who was chair of USPSTF when it made its mammography recommendations in 2009, says that what really surprised him was being shut out of the subsequent public debate. “It was frustrating to me not being able to counteract the very carefully crafted misinformation from the advocates” of mammography screening, says Calonge, now CEO of The Colorado Trust in Denver, a philanthropy. “We could not get control of the message.”

In its early days, USPSTF undertook two reviews of more than 150 preventive measures—trying to cover the universe, McGinnis says. But medicine was changing too rapidly to keep up this pace; in the late 1990s the panel switched to doing analyses of single topics, which it now selects with input from researchers and the public. This year, for example, more than 20 topics are under review, from screening for abdominal aortic aneurysms to the use of vitamin D supplements to prevent cancer and osteoporosis.

Calonge, Moyer, and McGinnis all say that USPSTF needs to learn to communi-

cate findings more clearly. And they agree on another point: A bill proposed in Congress this year to revamp USPSTF would not be a great way to improve scientific reviews. Introduced by Representative Marsha Blackburn (R-TN) and others, the USPSTF Transparency and Accountability Act of 2012 (H.R. 5998) would require the task force to include “stakeholders” and “specialty care providers” on decision-making panels. This is the plan backed by urological associations.

Moyer acknowledges that the task force can use advice from medical specialists, but only if they are experienced in clinical epidemiology and primary care—and are thoroughly impartial. Recruiting advocates as reviewers would defeat the purpose of the task force, she says.

Time is running out for the Blackburn bill in this Congress. But if it fails this year, the appeal to shake up USPSTF by including advocates may come back in 2013. *Science* recently spoke with Moyer about her role, her task force’s recent fame, and its future. Her answers have been edited for brevity and clarity.

—ELIOT MARSHALL

#### Q: How did you get into this field?

**V.M.:** I think I got into evidence-based medicine because I tend to say, why are we doing this? (I have a journalist father and a scientist mother.) I was extremely fortunate to go to an early workshop sponsored by McMaster University, one of the birthplaces of evidence-based medicine. I got very involved. ... I had written a paper about whether there was any evidence for what we do in pediatric checkups. I looked at procedures that were recommended by at least

two august bodies for these checkups and dug up the evidence for them, and it turns out there’s not a lot of it. That doesn’t mean they don’t work.

#### Q: On the task force, was there concern about potential harms from preventive measures from the beginning?

**V.M.:** Yes, this was a new way of looking at things. We don’t just say, “Oh, prevention is wonderful.” As in all of medicine, you have to look at the upsides and the downsides, the benefits and the harms. ... If you have someone who feels fine when they come into the office, and you give them a screening test, you can’t make them feel better. The only thing you can do is make them feel worse. So we balance both the benefits and the harms of any preventive service.

#### Q: Did a concern over cost drive this balancing act?


**V.M.:** That was not a big concern. It was more general. ... You don’t want to do something that doesn’t work—that is just a waste of money. More important, it is a waste of time. In a clinical encounter, you don’t want to waste any of the limited time you have.

#### Q: Why was the recommendation against routine mammography controversial?

**V.M.:** The timing of the release coincided with health reform and the election. People were looking for a reason to be upset. ... The health care reform bill said that if the USPSTF makes a recommendation with a grade of A or B, that service would be first-dollar covered [by health care providers]. Well, the task force didn’t give mammography for women aged 40 to 49 an A or B, so people interpreted it to mean they wouldn’t be able to get [insurance] coverage. But that isn’t what the recommendation says at all.

## Online

sciencemag.org

 Podcast interview with author Eliot Marshall ([http://scim.ag/pod\\_6101a](http://scim.ag/pod_6101a)).

### A TAXING EFFORT:

Rates of obesity, heart disease, and diabetes are all increasing worldwide, and many nutritionists lay the blame on eating too much food laden with sugar and fats. Last year, Denmark sought to curb poor nutrition by making certain products more expensive, introducing a tax adjusted to the saturated fat content on products such as butter, milk, cheese, pizza, meat, oil, and processed foods.



**THWARTING DECAY:** Cavities, or caries, result when an infection—usually bacterial—demineralizes teeth, but more than a century ago researchers began to explore whether fluoride in water could stymie that process. After a study in a Michigan city showed that fluoridating the water supply reduced tooth decay among residents, U.S. officials backed the prevention strategy in 1951. Water fluoridation would soon become widespread in the United States and many other countries, though some worry about its other health implications or argue it infringes their rights.



**Q: What did you take away from that experience?**

**V.M.:** We learned that we were suddenly in the public eye, which has dramatically changed how we communicate. We have expanded the transparency of what we do and opportunities for input from the public and from other stakeholders.

**Q: Did you have public comment before?**

**V.M.:** We did not. ... Everything went out for review to all kinds of people who we thought would be interested. Now it is put out on our Web site for anyone who wants to respond. We now invite public comment at the point when we are starting to think about a topic, so the public can say, "Well, you are not asking the right questions." ... It really helps us to understand what people are interested in, what they are concerned about.

And other experts may look at an issue and say, "I think you should frame it differently."

**Q: Did the PSA recommendation turn out to be as controversial as mammography?**

**V.M.:** In some ways it is probably more controversial. The mammography recommendation itself is not all that exciting. ... It says: Think carefully because there are some downsides. ... Don't just leap into it. The PSA recommendation says: Don't do it. That has been very controversial with the urologists and with some but not all advocacy organizations. ... We all have great

hope [that preventive screening will work], and it is pretty painful to have that hope taken away.

**Q: Have task force recommendations caused a change in behavior?**

**V.M.:** There has been a change in mammography frequency—that has been pretty well studied now over the last couple of years.

And there have been a couple of studies looking at the frequency of PSA [tests]. And [both] have gone down a little bit.

**Q: Does it take a long time for recommendations to be acknowledged?**

**V.M.:** Absolutely. We shouldn't be surprised by this. There are tons and tons of data out there on how long it takes for changes in health care to really take hold in practice. There was a study decades ago that's always quoted that says it takes

18 years to get anything new into practice—that may not be exactly accurate, but it is true that change is hard and it takes a long time.

**Q: There's a bill in Congress mandating that medical specialists be included on USPSTF panels. What do you think of it?**

**V.M.:** It is important to recognize that ... we [at USPSTF] already do have the appropriate specialists on the panel: specialists in primary care and in evaluating science. Our recommendations are addressed to primary care clinicians about people who visit their generalists without specific concerns; these

are *not* people who are going to specialists with a concern. If you put advocates on the panel, all you're doing is asking for advocacy, not science.

**Q: How much should task force work be linked to policy, such as spending decisions?**

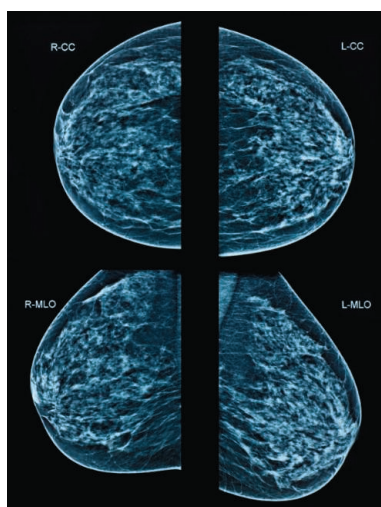
**V.M.:** In many countries—England is a good example—the body that does the type of work we do is directly linked to policy decisions. In this country, that doesn't go as well. ... We do not do cost-effectiveness analysis. That is a scientific field in and of itself, and it's very resource-intensive. We don't do that.

**Q: Some say it would be a mistake to link the scientific review and policy decisions because the science would suffer.**

**V.M.:** That tends to be right. ... We don't ignore the fact that there are [financial] costs associated with things, and we particularly consider cost to the individual to be a potential harm, but not in an explicit quantitative way. We do consider the fact that a false-positive test not only ends up requiring in many instances invasive and unpleasant procedures to determine that it was a false positive, but it can also be costly to the individual in time and money. My most recent false-positive mammogram cost me \$2000 out of pocket, because insurance only covers the mammogram; it doesn't cover the biopsy. Two thousand dollars is real money. Our purpose is not to save the system money. Our purpose is to improve the health of all Americans.

**Q: Will the task force always be in the middle of controversy?**

**V.M.:** Probably. And that's OK. Somebody needs to be looking at the things that we [physicians] always do with a critical eye.



**Screening shocker.** The task force's decision on mammography upset many groups.

**BABY SAFEGUARD:** There's frequently debate about whether a screening method saves lives, but few doubt the effectiveness of one widely used method: the phenylketonuria (PKU) test now given to newborns in many industrialized countries. Often part of an arsenal of other newborn assays, the PKU test checks whether an infant has a toxic buildup of the amino acid phenylalanine, which can lead to mental retardation and other medical problems. If PKU is detected in a newborn, the infant can be given a specialized diet and avoid most health effects.

**TOBACCO WARNING:** In response to a 1964 report on smoking by the Surgeon General, the United States became the first country to mandate health-hazard warnings for cigarettes and other tobacco products. Other countries have followed suit, and the warnings have recently become even more graphic and aggressive.



**FLOUR POWER:** The addition of folic acid to breads, grains, flour, and other food products in many industrialized countries has been credited with reducing the number of babies born with neural tube defects. The U.S. Food and Drug Administration announced in 1996 that it would begin requiring folic acid fortification in certain foods, and a few other countries have taken the same action. Similarly, niacin has been voluntarily added to enriched flour in some countries to help ward off pellagra, the sometimes fatal disease caused by a B vitamin deficiency.

# Will an Aspirin a Day Keep Cancer Away?

Data suggesting that regular aspirin use lowers cancer risk has accumulated to the point where some argue that it's time to recommend that many more people take the drug

**IN THE LATE 1970S, A SURGEON IN** Melbourne, Australia, wanted to figure out why his country had a relatively high rate of colorectal cancer. After he and colleagues interviewed more than 700 cancer patients and a comparable number of healthy people, they reported in 1987 and 1988 that Australians' penchant for beer, fatty foods, and red meat all seemed to predispose them to the disease. The researchers also found a surprising protective factor: People who regularly used aspirin were 40% less likely to develop colorectal cancer than those who didn't take the drug.

That first hint that the age-old headache remedy also blocks intestinal tumors helped spur a wave of research in animals and clinical trials that established that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) protect against colon cancer. And now, 2 decades later, aspirin is generating new excitement among cancer researchers. A series of studies from the United Kingdom in the last 2 years has offered the first evidence from placebo-controlled clinical trials that regularly taking low doses of aspirin wards off other types of cancer as well.

The studies, which tallied cancer cases among people who had been taking aspirin for years to prevent vascular events such as heart attack and stroke, found that death rates from several tumor types were as much as 37% lower. And even in the people who developed a cancer, taking aspirin seemed to slow the spread of tumors to other parts of the body. "It's just about the first proof of principle that a simple compound of any kind can reduce the risk of several cancers," says lead researcher Peter Rothwell of the University of Oxford in the United Kingdom.

These reports have raised the tantalizing possibility that aspirin could serve as the first anticancer drug for the general population. "It reshapes the debate about the risks and benefits of aspirin for cancer prevention," says colorectal cancer researcher Andrew Chan of Massachusetts General Hospital in Boston.

Because aspirin can cause stomach upset and dangerous internal bleeding, U.S. guidelines now recommend that only people at elevated risk for heart disease or stroke take low doses of the medicine, typically 81 milligrams a day. But Chan and others suggest that medical societies and policymakers should also consider aspirin's general cancer-fighting



**Versatile.** Aspirin may block cancer as well as vascular disease.

effects. Rothwell, who is 48, is so convinced by his team's data, for example, that he's begun taking aspirin daily even though he has no risk factors for vascular disease.

The new British aspirin studies are also fueling basic research on NSAIDs to ward off cancer, a field that lost momentum in the past decade when one NSAID, Vioxx, was pulled off the market because of safety concerns. Partly because of the U.K. results, U.S. National Cancer Institute (NCI) Director Harold Varmus last year added to his list of 24 "Provocative Questions" one that asks what is the mechanism by which aspirin and

other NSAIDs protect against cancer (see sidebar, p. 1472). NCI hopes the attempt to resolve the question—there are several competing theories—will lead to next-generation anticancer drugs and biomarkers showing who will respond to them.

But some cancer researchers say health policymakers needn't wait for a better aspirin and should advocate wider use of the current one. "This is a really extraordinary opportunity if everything bears out, because you have a drug that costs a penny a pill at a low dose, that can prevent the two major causes of death in the Western world," says epidemiologist Michael Thun of the American Cancer Society in Atlanta.

## Comeback

Thun's optimism is the latest high in the up-and-down story of NSAIDs as a cancer preventive. Aspirin and some other NSAIDs first bore out their promise in trials published starting in 2000 among people who had had precancerous colon polyps removed and others genetically prone to colorectal cancer. The drugs protected them from polyps and premalignant tumors that precede full-blown cancer. Such people are now sometimes advised by their doctors to take NSAIDs as an adjunct to surgery to prevent polyps from recurring.

Although epidemiological evidence has suggested that aspirin could have broader anticancer effects, those results aren't conclusive. They come from studies in which people answered questions about their past use of medications—a design prone to bias in part because memories aren't reliable. And hopes for aspirin fell in 2005 when a huge prospective study—a randomized controlled trial called the Women's Health Study (WHS)—failed to show a reduction in the incidence of cancer in nearly

40,000 women who took low-dose aspirin every other day for 10 years.

The field of NSAIDs for cancer prevention also suffered a black eye when two arthritis drugs developed to avoid the side effects of aspirin, the COX-2 inhibitors Vioxx and Celebrex, were tested in trials to prevent colon polyps. In one large trial, Vioxx cut the risk of polyps but raised the chances of heart attack so much that risk arguably outweighed the cancer benefit. Vioxx was pulled off the market in 2004 and Celebrex, although still used for preventing colorectal cancer in people at high risk of such disease, now carries a



“black box” warning label.

The pendulum began to swing back in favor of aspirin, however, when Rothwell, a neurologist, noticed that fewer overall cancers had occurred among patients in the treatment arm of randomized trials launched in the 1980s to test aspirin as a way to prevent stroke. Amassing details on the cancers from patients’ paper health records and U.K. health databases, his team initially confirmed aspirin’s ability to ward off colorectal cancers, reporting in 2007 that there were 24% fewer cases and 35% fewer deaths in the aspirin group after several years. Then in late 2010, they reported that patients taking aspirin at any dose daily for at least 5 years cut by 21% the long-term risk of dying from colorectal, gastrointestinal, esophageal, stomach, pancreatic, brain, lung, and prostate cancers.

Rothwell’s group expanded on these results in three papers published in *The Lancet* and *The Lancet Oncology* this spring. One, a meta-analysis of 51 randomized trials in which aspirin was taken daily, found 37% fewer deaths from cancers after

**Believer.** Peter Rothwell’s cancer studies convinced him to take a daily aspirin.

5 years. (The study came with a large caveat: It excluded WHS and an earlier trial that found no benefit, the Physicians’ Health Study, because participants in each took aspirin only every other day.) This meta-analysis also found that while the aspirin group had more stomach bleeds, these incidents were not fatal—people recovered—and the bleeding risk went down after several years on aspirin. Another paper found that people on aspirin who developed cancer were 36% less likely to have tumors that had spread. A third paper found a “remarkable consistency” in the drop in cancers among aspirin users in epidemiologic studies and clinical trials, Rothwell says.

While these studies weren’t designed specifically to test aspirin against cancer, some researchers say that because so many people now take aspirin off-label it would be diffi-



cult to conduct better trials. The Rothwell studies are “probably the best evidence we’ll ever have on this topic,” Chan says.

### Weighing risks

Chan is part of an international panel on cancer prevention that, in response

to the Rothwell studies, plans to update its stance on aspirin published 3 years ago. The group’s leader, epidemiologist Jack Cuzick of Queen Mary, University of London expects the panel may suggest that people take low doses of aspirin daily starting around age 50 and stopping by age 70, when the risk of internal bleeding rises. The group may also discuss whether doctors should screen patients for the ulcer-causing *Helicobacter pylori* bacterium, treating those who test positive with antibiotics before putting them on aspirin, to reduce the risk of bleeds.

Some U.S. researchers also suggest that it’s time for the U.S. Preventive Services

## Wondering How the Wonder Drug Works

**HOW DOES TAKING ASPIRIN WARD OFF CANCER?** RESEARCHERS still don’t understand the mechanism, a lack of knowledge that threatens to frustrate development of better cancer prevention agents. But some say the latest clinical studies linking low-dose aspirin use with less cancer (see main story, p. 1471) suggest there’s no need to improve upon this 113-year-old workhorse drug.

Among its many known effects, aspirin, or acetylsalicylic acid, inhibits two forms of enzymes known as cyclooxygenases (COX) that convert arachidonic acid into lipids called prostaglandins. Prostaglandins made by COX-1 protect the stomach lining, while those made by COX-2 are involved in inflammation. Aspirin’s inhibitor effects on COX-1 seem to explain why the medicine can upset stomachs and trigger internal bleeding. Many researchers have concluded that aspirin prevents cancer mainly by blocking the activity of COX-2. That’s because the same inflammation-driven responses that help tissue recovery from wound injury—cell division, blood vessel formation, and suppression of programmed cell death, for example—may also help tumors grow.

Several lines of evidence implicate COX-2 in cancer induction. The enzyme is overproduced in many cancer types; mice lacking its gene are less prone to colon cancer; and trials of nonsteroidal anti-inflammatory drugs (NSAIDs) that target only COX-2 protected against precancerous colon polyps in people at high risk.

Reducing inflammation via COX-2 probably isn’t the only way aspirin prevents cancer, however. “We are as a field getting down to some of the mechanisms, but it’s very complicated,” says Raymond Dubois of the MD Anderson Cancer Center in Houston, Texas. A decade ago, researchers found that aspirin can block production of a protein called

NF- $\kappa$ B that protects cells from early death. Although the studies were done in the test tube at aspirin doses much higher than those in the bloodstream, the NF- $\kappa$ B hypothesis remains on the table—along with more than a dozen possible mechanisms—from inhibiting various cell growth pathways to targeting cancer stem cells. “There are a number of very different, divergent ideas,” says Asad Umar of the National Cancer Institute’s Division of Cancer Prevention in Bethesda, Maryland.

Some experts say new clinical studies of low-dose aspirin and cancer suggest that COX-2 isn’t directly involved at all. That’s because at low doses, the drug doesn’t block COX-2 but still impairs platelets, the small cell fragments in blood that help form clots, via the COX-1 pathway. Studies suggest that platelets blunt the immune attack on cancer cells traveling in blood and produce growth factors that help them take root in a new place, notes University of Pennsylvania pharmacologist Garrett FitzGerald. Some experiments suggest that activated platelets can also stimulate the COX-2 pathway in adjacent cells, which would explain how aspirin, by damping COX-2 at the site of an injury, could block the early stages of colorectal cancer, says Carlo Patrono, a pharmacologist at the Catholic University in Rome, Italy.

Efforts to make an alternative to standard aspirin haven’t yet panned out. Several drugs that target only COX-2, notably Vioxx and Celebrex, unacceptably raised heart attack risk, for example. And a version of acetylsalicylic acid containing a chemical group called nitric oxide that was supposed to reduce side effects failed to gain regulatory approval. Some researchers are combining aspirin with other compounds such as a lipid to reduce stomach upset. But these are years from the clinic. For now, “I don’t think a better aspirin is the issue,” but rather figuring out which individuals stand to benefit from it most, FitzGerald says. —J.K.



Task Force (see p. 1468) to update its guidelines on the risks and benefits of daily aspirin use. The group had endorsed its preventive prowess for heart attack and stroke but discounted its anticancer effects. The potential to protect against both cancer and heart disease could tip the balance toward recommending aspirin for many more healthy adults, Thun says.

Others are more cautious about recommending broad use of aspirin to ward off can-

***"It reshapes the debate about the risk and benefits of aspirin for cancer prevention,"***

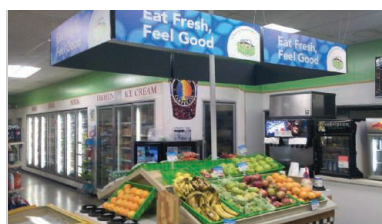
—ANDREW CHAN,  
MASSACHUSETTS  
GENERAL HOSPITAL

cer. "In 2012, gastroenterologists still get called to the emergency room for bleeds" caused by the drug, says cancer prevention researcher Andrew Dannenberg of Cornell University. Colorectal cancer researcher Sanford Markowitz of Case Western Reserve University in Cleveland, Ohio, points to studies suggesting that only people with a particular genetic profile will see their cancer risk go down if they take aspirin. "I would want more data on who benefits and who does not," Markowitz says.

Cancer prevention researcher Raymond Dubois of the MD Anderson Cancer Center in Houston, Texas, is also wary. "Initially we thought we could put aspirin in the drinking water. That's not the case," he says. "Where this field is going is towards a more personalized approach."

The picture may become clearer soon when WHS reports on longer-term effects of aspirin on cancer risk. Their next paper "will be crucial," Rothwell says. And even some on the pro-aspirin side urge caution. "We don't want to mess this up," Thun says.

—JOCELYN KAISER



**Philadelphia story.** With a push from a local nonprofit, The Food Trust, and a windfall of federal funding, efforts are underway to boost healthy offerings at small grocers around the city.

## Tackling America's Eating Habits, One Store at a Time

Several initiatives are evaluating if easing access to healthy food will change how Americans eat—and reduce obesity around the country

**PHILADELPHIA, PENNSYLVANIA**—The food market Clara Santos runs in South Philadelphia is less than 5 kilometers from the city's majestic art museum. Driving there, polished brick townhouses and trendy eateries with names like Caffeination and The Belgian Café give way to stretches of modest row houses, abandoned lots, and the occasional corner store advertising massive sandwiches called hoagies. Santos, who emigrated from the Dominican Republic, lives not far from the Olivares Food Market she took over 4 years ago. Graffiti marks its outer walls, but the shop, on a quiet block sandwiched

between two city schools, is tidy inside. Children stop in frequently for soda and candy after classes let out.

Santos's store is one of 642 data points in an unusual urban experiment. Eight years ago, a local nonprofit called The Food Trust began studying whether bringing healthy, affordable food to corner stores like hers could change eating habits among the city's economically disadvantaged residents. The program started small. Then in 2010, the Healthy Corner Store Initiative rapidly expanded after partnering with the Department of Public Health here, which had just received a windfall:

\$15 million to help prevent obesity, part of \$373 million from the federal stimulus package earmarked for health and wellness efforts around the country. Santos joined the project last year along with hundreds of other convenience store owners. Among other support, she received a display refrigerator where she showcases yogurt, precut watermelon, carrots, apples, and other fruits and vegetables, along with green bins for produce such as onions and plantains. Why not give the program a try, Santos says she thought: "Maybe we'll help the community."

Like Santos and those at The Food Trust, more and more people are trying to change the landscape of food in America. And change is desperately needed. More than one-third of



U.S. adults are obese, as are about 17% of children ages 2 and up. In 2008, researchers estimated that obesity cost the nation a staggering \$147 billion in medical costs.

Many paths lead to obesity, and the lack of access to healthy foods is thought to be just one of them—but for those on the front lines, it seems among the easiest and cheapest to modify. The strategy is also getting a boost from first lady Michelle Obama, whose plan to combat childhood obesity includes the elimination of “food deserts,” defined as low-income communities that are a significant distance from a full-fledged grocery store, though the specifics vary depending on whether the neighborhoods are urban or rural, among other factors.

Swapping food deserts for stores boasting fresh produce and other healthy foods—and using varied strategies in hopes of coaxing locals to buy them—comes with two questions. First, does promoting these foods change eating habits over the long haul? And second, does this, in turn, help fight obesity? Marketing bananas, demonstrating how to use cabbage in meals, and putting more boxes of wholesome cereals at eye level all sound like sensible practices that should help people lose weight or keep it off, but until recently, more effort has gone into initiating such interventions than scientifically measuring whether they matter. People “have an intuitive feel about the value and worth of this,” since individuals cannot improve their eating habits and potentially lose weight if the food isn’t available to them, says Allison Karpyn, the director of research and evaluation at The Food Trust. “The mandate from policymakers was, ‘These communities need stores, they need revitalized neighborhoods.’ It wasn’t, ‘We want to measure their BMI.’”

### Evolving tactics

In the early days of food-desert research, it was hoped that simply plunking down a well-stocked supermarket where there wasn’t one could change eating habits. One of the first people to test this was Neil Wrigley, an economic geographer and urban planner at the University of Southampton in the United Kingdom. Through his planning work,

Wrigley was well-connected to the retail industry, and in 1999 he learned that a sizable supermarket would soon open in an impoverished area of Leeds.

The neighborhood had high crime rates and many very young mothers. It was, Wrigley says, “isolated from the mainstream of economic life.” From this community, Wrigley recruited 1009 households to help him examine how eating habits changed after the supermarket opened. Tracking eating habits for a week, 5 months before and again 7 months after the supermarket opened, Wrigley determined that the new supermarket did indeed influence food choices, but its impact was slight and variable.

Taking together all 615 households that stuck with the study, there was no measurable difference in fruit and vegetable consumption. But when the researchers looked at only the

Another reason a new store has limited influence is that nearly everyone, no matter how they eat, is accustomed to buying roughly the same foods and preparing roughly the same dishes week after week. “You have to change patterns that have been ingrained over time,” says Helen Lee, who recently moved from the Public Policy Institute of California in San Francisco to the social policy research organization MDRC in Oakland, California.

Lee studied how proximity to grocery stores affected childhood obesity in about 3200 communities around the country. The result? “What children had access to in their residential environments didn’t predict who became overweight or who stayed overweight,” she says. In general, evidence suggesting that people eat better when there’s healthy food nearby “is really mixed,” says Lee, who worries that eliminating food deserts in the absence of broader structural changes to combat poverty, such as educational reform and job training, could be a waste of precious dollars.

Against this uncertain backdrop are widespread efforts to promote access to healthy foods among the poor. In both New York City and Philadelphia, residents who use \$5 worth of food stamps at a farmer’s market receive \$2 more to spend on fresh produce there.


In New York City, the Shop Healthy NYC initiative is, among other things, encouraging owners of more than 1000 of small corner stores called bodegas to stock produce and

other healthier foods, such as low-fat milk. But tracing what people are actually buying in bodegas hasn’t panned out. “The majority don’t have electronic cash registers” and don’t provide receipts, says Sabrina Baronberg, who helps oversee the program at the city’s Department of Health and Mental Hygiene. “We’ve tried a variety of mechanisms. ... It’s very difficult to track sales.”

The push for change despite limited data has divided public health experts. On the one hand, many say that it’s hard to see a downside to offering more fruits and vegetables, low-sodium products, and low-fat dairy options to disadvantaged communities. On the other, with limited public health money, Lee, Karpyn, and others say, it makes sense to understand which strategies work best,

## Online

**sciencemag.org**

 Podcast interview with author Jennifer Couzin-Frankel ([http://scim.ag/pod\\_6101b](http://scim.ag/pod_6101b)).



**Beacon.** This U.K. supermarket, which opened in an impoverished area in 2000, was studied for its impact on eating habits.

several hundred households that switched to shopping at the new store, there was a modest change: They added about a quarter-serving of produce to their diets, boosting the total to 2.89 servings—still far below the recommendations at the time of five servings a day. “We weren’t expecting a big impact from improving availability, we just wanted to see whether there was any impact,” says Wrigley, who called the results “heartening.”

Wrigley’s results suggested that a new store on its own has only so much power. Some consumers avoided the Leeds supermarket because they worried they’d be tempted by its rich offerings and would waste money. Furthermore, “many of these 21-year-old mothers had no experience in cooking at all,” Wrigley says.





rather than implementing a hodgepodge that may or may not make a difference. “I wonder if we’re going about it the wrong way,” Lee says, “if we’re putting the intervention before the science.”

### Store-by-store

In neighborhoods dotting San Diego County, Guadalupe Ayala is trying to put science first. Ayala, a public health researcher at San Diego State University in California, focuses on modifying behaviors to prevent obesity and other chronic health conditions. Currently, she’s working with 18 small-to-medium-sized grocery stores whose shoppers are mainly Latino. Nine of the stores are left alone. In the other nine, Ayala intervenes by helping the owners offer healthier foods: by providing, for example, a salad bar for precut vegetables (which many Mexicans like to eat, she says), or exploring how ingredients in prepared foods can be modified to make them healthier. The researchers are also providing marketing materials to highlight healthy foods. Thanks to their small size and the immigrant community they serve, the store’s staff members know many of their shoppers by name—leading Ayala to suspect that they might have sway over what customers buy. With this in mind, employees are trained “to do suggestive selling to promote healthier foods.”

To measure the outcome of the various interventions, Ayala and her colleagues are recruiting 396 customers—22 for each store—and tracking their eating habits for a year. The researchers are also studying what goes on in the stores: Do they sell more healthy foods? Do they allocate more space to such food? Do they use any marketing strategies beyond what Ayala suggests? To verify that customers are eating the fresh produce they buy, Ayala had planned to gather blood samples from 25% of participants and look at levels of carotenoids, a component in fruits and vegetables. But the grant, which is funded by the U.S. National

Cancer Institute, was reduced and that side project was eliminated. Still, Ayala has an impressive \$2.75 million for the effort—a sign of how expensive it is to probe these questions on even a small scale.

Another challenge for researchers like Ayala is keeping healthy foods affordable. She and her colleagues are keenly aware that if the foods they highlight aren’t roughly the same cost or cheaper than what they aim to replace, interventions are unlikely to work. This often means focusing on less expensive produce, such as tomatoes and zucchini. Or it might mean expanding the number of stores that belong to the federal government’s supplemental nutrition program for women and young children called WIC. Not every grocer that wants to can join WIC because of limited state resources to certify and monitor the stores. Those that do belong benefit from a guaranteed customer base. “We found that [participation in] WIC was the overriding determinant in whether a store had fresh fruits and vegetables, low-fat milk, [and] whole grains,” says Ann Ferris, who directs the University of Connecticut’s Center for Public Health and Health Policy in East Hartford. She has a paper coming out that backs this up.

To sell more healthy foods in Philadelphia, The Food Trust and its collaborators also rely on strategies used with great success to move junk food off store shelves. “If you put all the low-sodium goods together and put marketing around it and make it like a flashing beacon around the store, you’ll attract attention,” says Brianna Sandoval, who is overseeing the corner store initiative.

With colleagues at nearby Temple University and the University of Pennsylvania, The Food Trust is testing this approach in eight large grocery stores, most of them in Philadelphia. Four of the stores serve as controls, while in four others the researchers altered product displays in an effort to encourage healthier purchases. “It’s a stealth intervention,” says Gary Foster, who’s help-

**Selling the good.** Researchers and others are experimenting with marketing strategies to sell produce and other healthy foods, and steer patrons from unhealthy choices.

ing lead the study and directs the Center for Obesity Research and Education at Temple. “Where the whole milk used to be, the skim is.” More boxes of Cheerios are visible, along with more low-calorie soda; healthier frozen meals are at eye level. Still, Foster concedes that the approach is unlikely to radically change shopping habits. “In the scheme of things, it’s a pretty weak intervention,” he says. But because it costs stores little, it’s also sustainable.

As this store-by-store research progresses, there are glimmers that changing eating habits, at least a little, is doable. Interventions in San Diego, Baltimore, Philadelphia, and beyond suggest that customers can be coaxed into buying healthier foods. But even if this works on a grander scale, across urban and rural areas—and no one knows yet if it will—can such changes make a meaningful dent in obesity? “There’s an assumption built in here that nobody ever articulates,” says Kelly Brownell, director of the Rudd Center for Food Policy & Obesity at Yale University. The assumption is that healthy foods sit on one end of a seesaw, with unhealthy foods on the other—and thus that eating more broccoli and Greek yogurt means ingesting fewer McDonald’s hamburgers or liters of soda. There’s no evidence to support this, however, he says.

Brownell, whose favored strategy is making it more difficult to find and afford unhealthy foods, still believes that access to healthy food matters. More fruits and vegetables reduce cancer and heart attacks, and communities deserve food choice. “If I didn’t study obesity, but I studied cancer or heart disease, I’d be very happy about” these projects, he says. “But we can’t necessarily expect [them] to help the nation’s obesity problem.”

—JENNIFER COUZIN-FRANKEL



**Ounce of prevention.** Cod-liver oil has been given as preventative medicine for decades (*left*, British children in 1938 are presumably getting their spoonful). Now scientists are amassing tens of thousands of volunteers to test the preventive power of one of its ingredients: vitamin D.

# Uncertain Verdict as Vitamin D Goes On Trial

Boosting levels of vitamin D with supplements has been touted to prevent diseases, but many scientists say only clinical trials now in the works can confirm such hopes

IN 1848, DOCTORS AT THE HOSPITAL FOR Consumption and Diseases of the Chest in London undertook one of the world's first clinical trials. More than 1000 patients with tuberculosis (TB) were either just cared for, as no effective treatment was known, or were also given a spoonful of cod-liver oil three times a day. Nineteen percent of patients on cod-liver oil deteriorated or died, compared to 33% in the control group.

Before antibiotics became available in the mid-20th century, many TB patients were also sent to sanatoriums in Switzerland or other countries. Lying in their beds, they were wheeled out into the sun for phototherapy. Looking back, says Adrian Martineau, an immunologist at Barts and The London School of Medicine and Dentistry in London, the two experimental therapies had something in common: vitamin D, which wasn't discovered until 1922.

Unlike other vitamins, the human body produces most of its vitamin D itself—with the help of sunshine. In the skin, a precursor molecule called 7-dehydrocholesterol is turned into vitamin D<sub>3</sub> by UV light. Ninety percent of vitamin D circulating in the human body is produced that way. Only 10% comes from food, in the United States and Canada mostly from milk, which is fortified with the vitamin in those countries. It is also naturally abundant in some foods including fatty fish, sun-dried mushrooms—and cod-liver oil.

Martineau has studied the connection between TB and vitamin D for years and has become convinced that the compound can not only help treat TB but also prevent it. He is part of a vocal camp of scientists who praise the powers of vitamin D and see it as something of a cure-all—or rather a prevent-all. In addition to its well-established benefits for bone health, they say vitamin D may—with little or no side effects—be able to ward off colds and other infections and cut the risk of asthma, diabetes, cancer, heart disease, and a slew of other chronic diseases.

Some of these scientists, such as Michael Holick, an endocrinologist at Boston University School of Medicine and a veteran of vitamin D studies, advocate fortifying more foods with the vitamin and advising people to take supplements and get more sun exposure. “Even if only one of these diseases turns out to be prevented by vitamin D, it is worth it,” he says. The public certainly seems to buy that argument. In the United States, sales of vitamin D supplements have increased from \$50 million in 2005 to \$600 million in 2011, according to the *Nutrition Business Journal*. Ninety years after it was discovered, vitamin D seems to be enjoying its moment in the sun.

But other researchers warn that the benefits of vitamin D are far from proven. They also caution that its widespread use as a supplement could do more harm than good, as

trials of other vitamins have shown. Even a believer like Julian Peto, an epidemiologist at the London School of Hygiene and Tropical Medicine, cautions, “You have got to be very certain. Mass medication is not something you embark on lightly.” He adds: “What we know comes mainly from observational studies.”


In the next few years, however, the long-standing vitamin D debate may finally be put to rest. In a number of large clinical trials, tens of thousands of people around the world will take a supplement or placebo pills in an effort to pin down the health benefits of the sunshine vitamin.

## A body of evidence

Vitamin D was first recognized for its role in bone health. It helps the body absorb calcium, and children who do not get enough of it can develop rickets, a bone-softening disease. But low vitamin D levels have also been implicated in infectious diseases. For instance, patients with TB tend to have lower vitamin D levels, and Martineau points to studies showing that the compound helps immune cells called macrophages kill the mycobacterium responsible for TB, as well as suppress the secretion of enzymes the pathogen uses to degrade lung tissue.

In 2010, a randomized trial in 334 Japanese schoolchildren found that those taking vitamin D supplements were less likely to suffer from an influenza infection. And in August, scientists from Harvard Medical School in Boston reported in *Pediatrics* that Mongolian schoolchildren whose milk was fortified with vitamin D had half the risk of catching a cold compared to those drinking unfortified milk. It is perfectly plausible that lower vitamin D levels in winter might be the reason colds predominately circulate then, Peto says.

Proponents of the sunshine vitamin have also amassed a variety of data suggesting it wards off asthma, diabetes, stroke, multiple sclerosis, and cognitive decline. And a body of evidence indicates vitamin D could cut the risk of cancer and cardiovascular disease dramatically, they argue. For colorectal and breast cancer alone, raising vitamin D levels on a population level could pre-

 Related video on Vitamin D.  
[www.scim.ag/vitamin\\_vid](http://www.scim.ag/vitamin_vid)



vent more than 100,000 cases each year and cut deaths from these diseases by three-fourths in the United States and Canada, a paper published in the *Annals of Epidemiology* in 2009 calculated.

One comprehensive review of the vitamin D literature, a meta-analysis published in 2011 by the Cochrane Collaboration, concluded that vitamin D<sub>3</sub> supplementation (but not other forms of the vitamin) reduced overall mortality by about 6% among the more than 90,000 people in the 50 studies examined. “That is not overwhelming, but it is borderline significant,” says Robert Scragg, a vitamin D researcher at the University of Auckland in New Zealand.

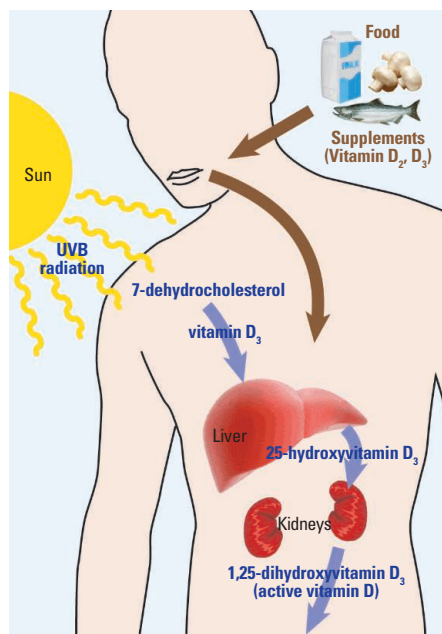
Many vitamin D enthusiasts point to evolution to bolster their case. Dark skin protects skin cells from UV damage, but it also reduces the amount of vitamin D that is produced; African-Americans in the United States generally have lower levels of vitamin D in the blood than the rest of the population. “The strongest single bit of evidence [that more vitamin D is good for a person] is that humans turned white when they moved north,” Peto says. “That suggests low vitamin D levels must have had [a negative] effect on survival.”

### Mass medication

However, some veteran vitamin researchers caution that other vitamins have been linked to a broad range of health benefits, only to have the evidence crumble upon closer examination. In the 1990s, for example, observational studies suggested that antioxidants such as beta carotene (a precursor of vitamin A) could shield the body from the cancer-causing compounds in tobacco and other harmful substances. But in 1994, a prospective clinical trial with nearly 30,000 smokers in Finland concluded that those who had taken beta carotene supplements were actually 18% more likely to develop lung cancer and 8% more likely to die during the trial. Two years later, a U.S. study examining vitamin A supplements in smokers and asbestos workers was stopped early because there were 28% more lung cancers and 17% more deaths in the group receiving



**Vital sunshine.** UV rays striking the skin convert the molecule 7-dehydrocholesterol to an inactive form of vitamin D<sub>3</sub>. It is then converted first in the liver and then the kidneys to 1,25-dihydroxyvitamin D<sub>3</sub>, the active form of the vitamin.



vitamin A than among the untreated.

Another antioxidant, vitamin E, was also touted as a cancer killer. But in 2008, a cancer-prevention trial evaluating vitamin E and selenium supplementation was stopped because participants taking vitamin E had become more likely to get prostate cancer. The risk difference then was not statistically significant, but follow-up data published late last year showed a significant increase of 17% compared with the control group.

Some predict that history is about to repeat itself. “I think vitamin D is going the way of these other treatments,” says Andrew Grey, a researcher at the University of Auckland. Low levels of vitamin D might simply be a marker of bad health rather than the cause of it, he suggests: “Almost always the levels are lower in patients who are sicker, but that could be because they exercise less and do not go outside so much.” Another confounding factor: Vitamin D is fat-soluble, so obese patients also tend to have lower levels of circulating vitamin D.

JoAnn Manson, an endocrinologist at Harvard Medical School, agrees that once again enthusiasm for a vitamin is outpacing the evidence: “There are many reasons that low vitamin D levels might be linked to these chronic diseases. Correlation does not prove causation.”

Other groups reviewing vitamin D data haven’t been as impressed as the Cochrane group. After sifting through hundreds of studies, a panel convened by the Institute of Medicine (IOM) concluded in 2010 that vitamin D was important for bone health, but that evidence did not support other benefits from vitamin D intake.

### Difficult dosing

Tackling another contentious issue at the heart of the vitamin D debate, the IOM report also recommended an adequate blood level of the vitamin: 50 nmol/l. “Some people with malabsorption may need higher levels, but for the healthy population 50 nmol per liter is certainly enough,” says Clifford Rosen, a bone-health expert at Maine Medical Center Research Institute in Scarborough, who was on the panel. The report also pointed out that most people in the United States reach that level through diet and sun exposure alone.

The strong vitamin D proponents, as well as other scientists, say the IOM threshold is too low and hark back to prehistoric times to make their point. They argue that as humans started wearing clothes, developed sunscreens, and began spending many hours indoors, they cut themselves off from the level of vitamin D they used to have. Dutch scientists published a study earlier this year examining vitamin D levels in two tribes in Tanzania. Living close to the equator, following a hunter-gatherer-like lifestyle, and not using sunscreen, the Maasai and Hazabe peoples had a mean serum concentration of 115 nmol/l. “That is probably where we all should be,” says Holick, who takes supplement pills to keep his vitamin D level between 100 and 150 nmol per liter.

Others don’t aim as high, at least for keeping the skeleton strong—the best studied aspect of vitamin D prevention science. “To ensure good bone health in everyone, you need to aim for a level of 75 nmol per liter,” says Michael Amling of the University Medical Center Hamburg-Eppendorf in Germany.

In a 2010 paper, Amling examined the bones and vitamin D levels of 675 people who had died in car accidents or of other unnatural causes. Seven of 82 people with a level above 50 nmol/l had weak bones. “That means almost 10% of the people with a serum level above this threshold have weak bones,” Amling says.

But the IOM panel, which had set itself the goal of allowing no more than 2.5% of the population to be at risk of brittle bones, used a different number: It divided the seven bodies with high vitamin D but low bone health by the total number of bodies: 675. “That was a grave mathematical mistake,”





Amling says.

Rosen warns that having higher blood levels of vitamin D could be harmful. "I can actually live with 75 nmol per liter, but above that I am a little concerned," he says.

He and others cite a 2010 Australian study in which women aged 70 years or older were given a megadose of 500,000 international units (IUs) once a year. Vitamin D levels in their blood shot up to an average of 120 nmol/l, but these participants also fell and fractured their bones more often than those in the placebo group, the scientists reported.

Proponents of vitamin D argue that such a megadose is unphysiological and that the study is a special case that should not be weighed too heavily in any risk-benefit anal-

### Proving prevention

With the intent of resolving the usefulness of vitamin D, several investigators are launching large-scale trials to examine the benefits of vitamin D in a number of chronic diseases. In the VITAL study Harvard's Manson leads, for example, 20,000 healthy people in the United States will receive either 2000 IUs of vitamin D a day or a placebo for 5 years. That should be enough to raise blood levels of vitamin D to 75 nmol/l or more in nearly all participants, she says. The trial will look at health outcomes as diverse as stroke, diabetes, cardiovascular disease, and cancer. The first participants are already taking their pills, and the last ones should be recruited by the end of the year, Manson says. Similar studies are starting in Finland, New Zealand, and Switzerland;

the volunteers will be mailed 400 pills once a year. If many people forget to take their pills, that would make it harder to detect a difference between the treatment and control groups.

There is another respect in which the vitamin D trials differ from most other randomized clinical trials: Normally, the placebo group receives none of the compound being investigated, but participants in the placebo group of a vitamin D trial will still produce the vitamin in their skin and consume it with their food. That narrows the gap between the two groups. In addition, in all the ongoing trials, participants will be allowed to take low-dosed vitamin D supplements if they were already taking them. Holick sees that as a fatal flaw in the VITAL study and others: "They are essentially comparing 800 IUs a day with 2000 IUs." The trial should have given the treatment group 4000-IU supplements to see a clear difference between it and the "control" group, he argues. But Manson points out that participants in both groups are allowed to take supplements. "The difference remains 2000 IUs," she says.

The double-blind Finnish study, which will start in a few months, will divide participants into three groups of 6000 that will take either a 1600-IU vitamin D supplement daily, 3200 IUs, or a placebo. And in the study in New Zealand, which in 2017 could be the first to report results, participants will take 100,000 IUs or a placebo once a month.

Even these large studies may not be definitive enough on their own to settle the vitamin D issue, says Scragg, who heads the New Zealand study. He cites recent evidence suggesting that vitamin D may only be beneficial in those with low initial levels of the vitamin. That means proof of the effectiveness of supplementation may only come from pooling the various studies, he says: "Then you could segment people into various vitamin D ranges based on baseline levels and see whether it has an effect or not."

Until those data are in, and maybe even afterward, scientists will likely keep on arguing, Rosen says. Evidence does not matter to many people when it comes to vitamin D, he maintains: "It is a religion. People really believe this stuff works."

—KAI KUPFERSCHMIDT

### Vitamin D on Trial

NAME	PLACE	PARTICIPANTS	DOSE	MAIN OUTCOMES	CURRENT STATE	RESULTS EXPECTED
VITAL	U.S.	20,000, men: 50+ women: 55+	2000 IU D <sub>3</sub> daily	Cancer, Cardiovascular disease	Recruitment to finish end of 2012	2017
FIND	Finland	18,000 men: 60+, women: 65+	1600 IU D <sub>3</sub> daily or 3200 IU D <sub>3</sub> daily	Cancer, Cardiovascular disease, Diabetes	Recruitment started in spring, supplementation to start in autumn	2020
ViDA	New Zealand	5100, 50+	100,000 IU D <sub>3</sub> a month (200,000 IU in June)	Cardiovascular disease, Respiratory disease, Fractures	Recruitment to finish this year	2017
DOHealth	8 European cities	2150, 70+	2000 IU D <sub>3</sub> daily	Infections, Fractures, Blood pressure, Cognitive function, Lower extremity function	Recruiting	2017
VIDAL	U.K.	20,000, 65–84	60,000 IU monthly	Longevity and others	Planned 2-year feasibility study on 1600 patients is recruiting	2020 (if main study gets go-ahead)

ysis. Rosen disagrees: "There is very little randomized clinical trial data that gets up to these levels, and there is just no evidence that it actually protects against skeletal problems or other diseases."

Interjecting another note of caution, a paper published in the *American Journal of Cardiology* in January 2012 showed that vitamin D in the blood reduces inflammation, measured by a protein called C-reactive protein (CRP)—until the vitamin's level reaches 50 nmol/l. Above that mark, the relationship reverses and more vitamin D increases CRP levels again. The authors concluded that supplementation with vitamin D to reduce inflammation may be beneficial only in those with low serum concentrations of the vitamin.

another one in the United Kingdom is in the pipeline (see table).

Such prevention trials are challenging—and may not ultimately satisfy everyone. They must have large numbers of subjects and run for a long time, because enough participants need to develop a disease to see a difference between the two groups. That makes them very costly; the VITAL study, funded by the U.S. National Institutes of Health, will cost about \$30 million. Compliance is also an issue, because healthy people taking part in a trial of vitamin D may be more likely to forget to take the pills than sick patients in a drug trial—especially because there is no doctor administering the treatment in a clinic. In the VITAL study, participants will receive their pills in the mail once a month; in the Finnish study,



## Chronic Disease Vaccines Need Shot in the Arm

Whether vaccines to prevent obesity, asthma, smoking-related illnesses, and other chronic diseases will ever work remains an open question

**IN JULY, GLOBAL HEADLINES PROCLAIMED** that a “flab jab” was imminent, an obesity vaccine that would allow consumption of endless fast and fatty foods without punishing weight gain. The proof: Mice that were injected with a vaccine targeting the hormone somatostatin gorged on a high-fat diet with significantly less weight gain than those given a sham injection. “Thousands of people contacted us volunteering for clinical trials. Everyone from mothers wanting to lose baby fat to weight lifters in Germany,” says vaccinologist Keith Haffer of small, South Dakota-based Braasch Biotech, who led the rodent study.

With funding interest from several South American companies, Braasch Biotech does plan to begin human clinical trials of its somatostatin vaccine late in 2013. Hold the extra cheese on that large sausage pizza, however. It’s difficult enough to develop and obtain approval for a traditional vaccine against a bacterium or virus, let alone create one that rouses the immune system to target molecules that drive a chronic disease such as obesity. Indeed, potential vaccines for hypertension, asthma, Alzheimer’s disease, obesity, and smoking (because it is a risk factor for heart disease, cancer, stroke, and more) have all been hyped for their promise in recent years and then suffered high-profile failures.

In 2002, for example, a vaccine that raised antibodies to the  $\beta$ -amyloid protein that accumulates in the brains of people with Alzheimer’s disease suffered a scary setback when

6% (18 of 298) of the clinical trial subjects receiving the AN1792 vaccine developed a severe brain inflammation. And Nabi Pharmaceuticals’s nicotine vaccine, designed to suck the high out of smoking, failed in a Phase III trial last year. Switzerland-based Cytos Biotechnology also attempted vaccines for smoking, as well as hypertension and type 2 diabetes, and though its researchers made progress, the company ran out of funding in 2011 and abandoned most of those efforts.

Vaccine developers also face a “psychosocial” problem, says Kim Janda, a chemist at the Scripps Research Institute in San Diego, California, who has worked on vaccines for

obesity, smoking, and addictive drugs for the past 30 years. “In large part, society still views addiction or even obesity as a moral failure rather than a chronic disease.” It’s difficult to persuade drug companies and the general population to invest in treating something they view as a failure of willpower with an intervention like a vaccine, Janda says.

But he isn’t giving up and neither are others. “If you can find a target that is the underlying cause” of an illness, Janda says, “then you can develop a vaccine for its treatment.” There are now a number of vaccines in clinical trials for cancer, which is considered a chronic disease, Janda says, so why not ones for obesity, diabetes, and drug abuse?

“We know why we failed previously and that there are clear pathways in front of us,” adds Martin Bachmann, an immunologist formerly with Cytos Biotechnology. He says that poor antibody responses and lack of specificity—critical flaws for a vaccine—are problems that companies are now addressing by using full-length proteins, rather than peptides, and experimenting with viruslike particles that yield a higher and more consistent antibody response.

### Mite-y vaccine

Vaccines were originally developed to combat microbial pathogens such as the smallpox virus and the tuberculosis bacterium; people are traditionally injected with live or dead copies of a pathogenic microbe, or with its molecular components—a viral surface protein, for example—to rally the immune system to produce antibodies or cells that specifically target the invader for destruction.

Yet vaccines may be able to do more than prevent infections. Consider asthma, the target of one vaccine effort. Worldwide, more than 300 million people suffer from asthma, often sparked by a violent immune response to common environmental allergens. Current treatments include corticosteroids, which reduce inflammation but have side effects, and a procedure called desensitization in which asthma/allergy patients are given increasing doses of an allergen cocktail. But the success of desensitization varies from person to person and occasionally causes a life-threatening reaction: anaphylaxis.

A team led by Bruno Pitard at the University of Nantes in France is now tackling asthma with a



**Mite-y vaccine.** Dust mites such as *Dermatophagoides farinae* (pictured) are a trigger of allergies and asthma, prompting efforts to immunize people against one of their proteins.

variation on the traditional vaccine. Pitard's strategy stems from the observation that 50% of Europeans with allergies harbor antibodies against the Der f 1 protein from *Dermatophagoides farinae*, one of the most common dust mites in the United States and Europe. But instead of immunizing with actual Der f 1 proteins from this mite, which can trigger an allergic response in people with asthma, Pitard and his colleagues are testing a vaccine composed of DNA coding for the protein, with the idea that it would train the immune system to tolerate it.

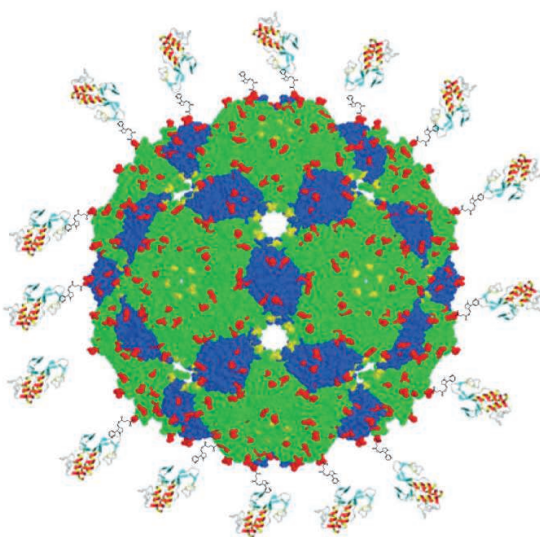
When naked DNA is injected into the body, however, it is rapidly degraded before it can express the antigen it encodes. That problem has frustrated many developing naked DNA vaccines, so Pitard is now treating his Der f 1 DNA with so-called tetrafunctional block copolymers, which, as his team discovered in 2009, encourage gene delivery into the skeletal muscle, where the protein can then be manufactured. The team recently immunized mice using this modified vaccine, and those mice had a fivefold reduction in their asthmatic reaction, Pitard says. Lung tissue and bronchioles carried far fewer inflammatory cells and cytokines than in asthmatic mice vaccinated with a placebo. Despite that encouraging data, it will be at least 5 years before a clinical trial of the asthma vaccine begins, Pitard predicts.

Most candidate vaccines for chronic diseases don't target microbial molecules but proteins made by the human body. Take the strategy behind the original Alzheimer's disease vaccine, which sought to activate the immune system against  $\beta$  amyloid. Because of the dangerous brain inflammation that followed, companies have largely turned to a so-called passive immunization, an approach in which they create antibodies targeting  $\beta$  amyloid outside the body, then inject them (*Science*, 17 August, p. 790). But several companies are still studying vaccines against different forms of  $\beta$  amyloid, which they hope will not produce the same side effects.

The various obesity vaccines also go after natural human proteins. Somatostatin, a small peptide hormone produced in the hypothalamus, inhibits growth hormone and insulin-like growth factor, which increase metabolism. The interest in a somatostatin vaccine for humans began when Haffer was

searching for another use for Braasch Biotech's vaccine Somatovac, which they found was a promising way to boost milk production in cows and lean meat production in pigs without using bovine growth hormone or antibiotics. He realized that Somatovac might also promote leanness in humans and therefore fight obesity.

Haffer sent two different versions of the



**Vaccine recipe.** First produce a viruslike particle, then chemically link multiple copies of an antigen, such as nicotine. The result: a conjugate vaccine that triggers strong antibody production.

somatostatin vaccine to Jackson Laboratory in Bar Harbor, Maine, where researchers there tested them on mice that had previously bulked up from consuming a high-fat diet for 8 weeks. Neither vaccine made the already-plump rodents lose weight, but they gained 10% less weight than control rodents, even though all the animals ate the same quantity of high-fat food during the 6-week study, Haffer reported in July in the *Journal of Animal Science and Biotechnology*.

The concept of creating vaccines for diseases like obesity isn't outlandish, but choosing the target is tricky, says George Jackson of the University of Texas Medical Branch in Galveston, a neuroscientist who is working on a next-generation vaccine for Alzheimer's disease. With Alzheimer's, he points out, you could potentially "target Mr. Hyde without harming Dr. Jekyll" because the culprit—a rogue form of  $\beta$  amyloid—appears to have no beneficial role. Obesity is different, he says: "Somatostatin is doing something bad but also something good. A vaccine could cause side effects by interfering with that normal function." Immunizing against an endog-

enous hormone controlling appetite might cause anorexia or wasting away, for example.

Haffer isn't the first to dream of an obesity vaccine. Janda and others have targeted the appetite-stimulating hormone ghrelin in their attempts to build an obesity vaccine. Back in 2006, Janda made headlines similar to those received by Haffer's work when he and colleagues reported that vaccinating adult male rats with a segment of ghrelin protein, or the full-length version, slowed weight gain and fat buildup in the body but didn't seem to affect appetite. Janda cautioned at the time that the study rats dined on a mundane, low-fat, low-energy chow. He couldn't predict from this study whether the vaccine would prevent diet-induced obesity—the kind that afflicts many people consuming a high-fat Western diet—or trigger weight loss in animals that were already obese.

Mariana Monteiro, an endocrinologist at the University of Porto in Portugal, presented more evidence at The Endocrine Society's Annual Meeting in June 2011 that an antighrelin vaccine could reduce appetite-promoting brain chemicals in mice. She revealed that tethering ghrelin to a viral protein could trigger enough antighrelin antibodies in the rodents to reduce eating, increase energy use, and reduce levels of neuropeptide Y—a potent appetite stimulator. But there was no overall weight loss at the end of the study. Monteiro wrote in October 2011 in *Expert Review of Vaccines* that the underwhelming impact on long-term food intake and body weight "might be due to activation of compensatory mechanisms."

Janda and his team have also explored passive immunization: injecting antighrelin antibodies in mice. In a study published in February in *Molecular Pharmacology*, Janda's team showed that a cocktail of three monoclonal antibodies targeting ghrelin could curb appetite and increase energy use. While this isn't really a ghrelin vaccine because it bypasses the immune system, it similarly "protects" the mice from the hormone and dulls its role as an appetite stimulant.

Even with expanding numbers of overweight and obese people worldwide, Janda's results so far haven't secured him National Institutes of Health funding to further develop a ghrelin vaccine. He's skeptical himself about tackling obesity this way. "I don't think there is one controlling molecule for metabolism," he says. "It's not going to be a panacea for all obesity."

Bachmann suspects that trying to develop a vaccine for obesity is hopeless. "It's so com-



plicated,” he says, “and people love to eat.” An obesity vaccine is fighting all the evolutionary safeguards that encourage an animal to eat and ward off starvation. He’s also not convinced that mice are a reliable indicator of whether an obesity vaccine will work in humans, noting that “every animal has different feeding behavior.”

### Nullifying nicotine

Bachmann remains more optimistic about a potential nicotine vaccine that could help people stop smoking, even though those efforts, too, have stumbled. A nicotine vaccine is subtly different from—and potentially safer than—those targeting obesity-related molecules or Alzheimer’s disease protein. The strategy, as with other so-called addiction vaccines for heroin and cocaine, is to create enough antinicotine antibodies in the blood to diminish the amount of the compound that makes it to the brain, in theory making an individual cigarette less appealing. Nicotine isn’t normally in the body, so there arguably should be less risk of side effects from inducing antibodies that block it.

In the late 1990s, scientists at Nabi developed and began testing NicVAX, a traditional conjugate vaccine that tethered nicotine, which by itself is so small that it’s invisible to the immune system, to a readily detected bacterial protein. Animal studies were promising: The vaccine stimulated the production of antibodies that bound much of the nicotine in the blood before it reached the brain, suppressing the pleasurable nicotine high.

The initial clinical trials examining NicVAX’s safety revealed no significant concerns, and by late 2007 Nabi announced that additional Phase II trials had allowed it to zero in on the most effective vaccine dose. The company also found that among the vaccinated smokers, the antibody response correlated closely with the ability to quit and remain abstinent. Late last year, however, the happy ending did not materialize. Nabi announced that its Phase III trial of NicVAX was a failure: There wasn’t a significant difference between the percentage of quitters in the NicVAX group and the placebo group.

Others are also finding an anti-smoking vaccine elusive. In 2008, Bachmann and his colleagues at Cytos tested their own nicotine vaccine in 341 smokers in a 6-month randomized, controlled Phase II clinical trial; 229 were given the vaccine while 112 received the placebo, administered monthly. After 2 months, the number of quitters in

the vaccine group was significantly higher than the placebo group, 47% versus 35%.

But after 6 months, the difference between the two groups was negligible. A closer look at the data offered some hope: The “high responders,” the people who produced the highest levels of antinicotine antibody in response to the vaccine, enjoyed the greatest success. Of the high responders, 57% had abstained from smoking after 6 months, compared with 31% of the placebo group. At 1 year, the numbers dropped to 41% and 21%, respectively, which Bachmann calls “statistically and clinically significant.” “Only one-third of the patients had a high antibody response,” he says. “If we could improve that by a factor of 3, then we might have a product.”

Vaccine development is an expensive game, however, and Cytos ran out of money a year ago. Bachmann has since launched two companies, Areba and Saiba, which focus on vaccines for Parkinson’s disease, malaria, and Alzheimer’s disease. “I don’t have the money to do smoking,” he says, though he is still a “great believer” in the nicotine vaccine.

Producing enough antibodies to bind to the 500 micrograms of nicotine in a cigarette before it crosses the blood-brain barrier in 6 to 10 seconds is a formidable hurdle. One radical strategy to meet that challenge, described on 27 June in *Science Translational Medicine*, is more akin to gene therapy than a vaccine. Janda and collaborator Ronald Crystal, a pulmonologist and genetic medicine expert at Weill Cornell Medical College in New York City, injected mice with a virus that travels to the liver, carrying the gene for a monoclonal antibody with a high affinity for nicotine. There, infected cells release antinicotine antibodies into the blood.

“When you give nicotine to mice, they chill out like people,” Crystal says. Their blood pressure and heart rate drop by almost

half within 25 minutes. Mice that received the team’s “vaccine” were unaffected when later given nicotine, running around with no change in blood pressure or heart rate. When Crystal’s team analyzed the animals’ blood samples, they found that 83% of nicotine in the serum was bound to the antibodies made by the inserted gene, preventing it from reaching the brain and triggering the dopamine reward system. In the brain, nicotine concentrations were just 15% of that in untreated control animals.

“It’s a second-generation vaccine for addictive molecules. It’s more elegant. It produces more antibody,” Crystal says, adding that the virus carrying the monoclonal antibody gene is currently being tested in another gene therapy clinical trial, so he’s confident of its safety record.

A nicotine vaccine interests Big Pharma, Janda notes, “because there’s money in it.” Smoking causes about one in five deaths in the United States each year, is responsible for 90% of lung cancer in men and 80% in women, and boosts the risk of stroke and heart disease two-fold to fourfold.

Pharmaceutical companies may indeed see a big market for a nicotine vaccine, but they’re not sure anyone can deliver a safe, effective product. “Novartis has made some contracts with biotech companies to develop antismoking vaccines,” says Rino Rappuoli, global head of vaccines research for Novartis Vaccines and Diagnostics in Siena, Italy, “but there has not been much progress. Big companies rely on biotech to derisk the sector.” As the stumbling efforts to

immunize people from Alzheimer’s, obesity, and smoking attest, however, developing vaccines for chronic diseases remains a risky proposition.

—BIJAL TRIVEDI

Bijal Trivedi is a freelance writer in Washington, D.C.



**An expanding epidemic.** A vaccine that could reduce weight gain would have a domino effect on other chronic conditions such as coronary heart disease, type 2 diabetes, and high blood pressure.

REVIEW

# Can Noncommunicable Diseases Be Prevented? Lessons from Studies of Populations and Individuals

Majid Ezzati\* and Elio Riboli

Noncommunicable diseases (NCDs)—mainly cancers, cardiovascular diseases, diabetes, and chronic respiratory diseases—are responsible for about two-thirds of deaths worldwide, mostly in low- and middle-income countries. There is an urgent need for policies and strategies that prevent NCDs by reducing their major risk factors. Effective approaches for large-scale NCD prevention include comprehensive tobacco and alcohol control through taxes and regulation of sales and advertising; reducing dietary salt, unhealthy fats, and sugars through regulation and well-designed public education; increasing the consumption of fresh fruits and vegetables, healthy fats, and whole grains by lowering prices and improving availability; and implementing a universal, effective, and equitable primary-care system that reduces NCD risk factors, including cardiometabolic risk factors and infections that are precursors to NCDs, through clinical interventions.

Improvements in sanitation, housing, and nutrition, as well as better treatment, have lowered death rates from infectious diseases such as diarrhea, pneumonia, and tuberculosis in most countries. This success has in turn increased the relative importance of noncommunicable diseases (NCDs)—mainly cancers, cardiovascular diseases (CVDs), diabetes, and chronic respiratory diseases—as causes of death (1). NCDs are now responsible for more than 35 million annual deaths in the world; more than 80% of these deaths occur in low- and middle-income countries (2). Medical interventions have improved the survival of patients with heart disease, stroke, breast cancer, and some other NCDs, but others like lung cancer still have high case fatality. Even when treatment is technically feasible, timely diagnosis and treatment require medical personnel, facilities, and medicines that are either lacking or costly, especially in low- and middle-income countries. Therefore, there is an urgent need for policies and strategies that help prevent NCDs. These policies and strategies should be guided by an understanding of how much, and through what specific actions, NCDs may be prevented or postponed to older ages.

Evidence on NCD preventability comes from data and analyses at two scales: individuals and populations. Studying individuals helps identify and establish risk factors that causally affect NCDs, and hence points to specific tools for disease prevention, but provides little information on how effective each of these tools may be in

disease prevention at the population level because the latter depends on the prevalence of risk factors in the population. Comparison of disease rates across populations or over time, especially when done in relation to risk-factor levels in the population, indicates by how much disease may be prevented and what the most important risk factors are at the population level. Here, we use examples of a number of NCDs and their key risk factors to make a case that we have sufficient knowledge from individual and population studies to substantially reduce the global NCD burden through prevention using a relatively small and coherent set of actions related to major risk factors.

## Modifiable Risk Factors and Cancers: Prevention of Diseases with a Few Dominant Risk Factors

Following the seminal work of Doll and Peto (3), researchers have used individual- and population-level data to examine the extent to which cancers can be prevented and identify interventions and strategies for doing so. The case of lung cancer dramatically illustrates how individual and population studies have come together to leverage a modifiable risk factor for effective prevention of a disease with low survival. We now know that about 70% of lung cancer deaths worldwide are due to smoking (Fig. 1) and would be prevented if people did not smoke (4). For men living in industrialized countries, more of whom have smoked and for longer periods, the proportion of lung cancer deaths due to smoking is even greater—over 90%.

The association between tobacco smoking and lung cancer was first noted nearly a century ago (5, 6). Subsequent studies, especially the work Hill, Doll, and Peto in the British Doctors Study (7, 8), gathered more detailed data on smoking behaviors and disease status and showed

that those who smoke from early adult ages are at least 20 times as likely as those who never smoke to die of lung cancer; the increased risk reaches 40-fold for heavy smokers who smoke about two packs of cigarettes per day (9).

Mirroring the high lung cancer risk in individual smokers, lung cancer death rates vary substantially across populations and over time based on smoking histories. In adult men, lung cancer death rates increased for much of the 20th century and peaked at ~170 to 190 per 100,000 (10) in a few Western European countries and the United States and subsequently in Central and Eastern European countries like Hungary (Fig. 2A). These peaks in lung cancer death rates tracked the peak of the smoking epidemic with a lag of about two decades. In women, lung cancer death rates in the majority of countries were less than 10 per 100,000 in the 1950s, virtually the same as men and women in Western countries who have never smoked (11). Women's lung cancer mortality began to increase two to three decades after that of men, reflecting the rise in women's smoking after World War II, first in English-speaking high-income countries and then in continental Europe. Owing to effective tobacco control and the decline in women's smoking, lung cancer death rates in women appear to have peaked in English-speaking countries, for example, at ~60 per 100,000 in the late 1990s in the United States (Fig. 2B). The situation is not all good, however, and lung cancer mortality continues to increase in women in Continental Europe. For example, the lung cancer death rate of Danish women, who have smoked longer and more than women in other European countries, is now higher than that of American women.



**Fig. 1.** Tobacco smoking is the most important risk factor for lung cancer and also has harmful effects on other NCDs.

MRC-HPA, Centre for Environment and Health and Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London W2 1PG, UK.

\*To whom correspondence should be addressed. E-mail: majid.ezzati@imperial.ac.uk



Although smoking is clearly the dominant modifiable risk factor for lung cancer, in certain populations other risk factors play a major role. People exposed to asbestos or to second-hand cigarette smoke are more likely to develop lung cancer than those who work and live in cleaner environments. In some parts of China, where coal is commonly used for cooking and heating in poorly ventilated homes, lung cancer mortality of people who have never smoked is about 4 to 5 times as high as that of those in Western countries who have never smoked (12). These examples illustrate the important point that the most effective disease prevention strategies are those that take into account the prevalent risk factors in the target population and by how much reducing any combination of these risks may lower disease levels. Patterns of lung cancer and its risk factors across the world and over time nonetheless demonstrate that stopping smoking, and a few environmental interventions in specific places, can reduce lung cancer to very low levels in every population.

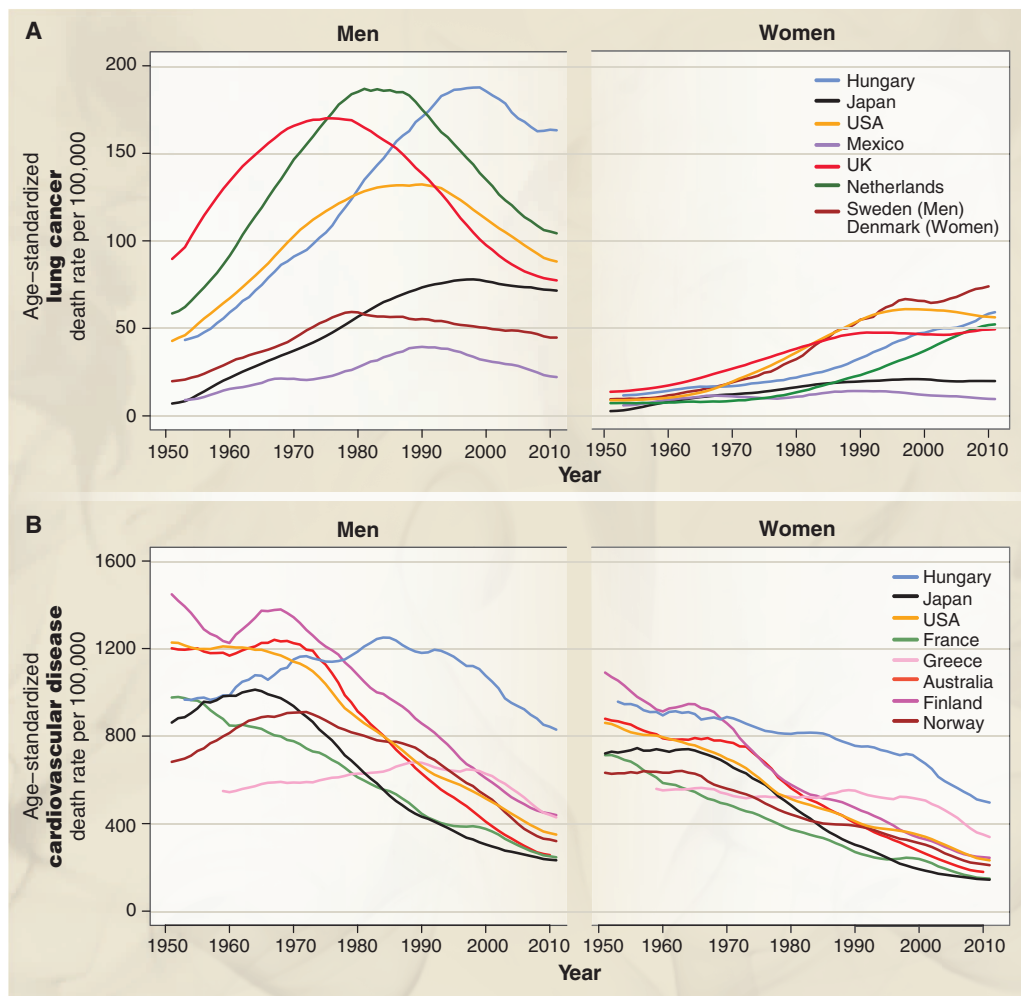
Dominant risk factors have also been identified for a few other cancer types, many in the form of an infection. In each case, these risk factors influence the geographical patterns and trends of these cancers and can be translated into effective prevention strategies and programs. For example, the discovery that *Helicobacter pylori* (*H. pylori*), a bacterium present in the gastrointestinal tract, is a risk factor for lesions that are precursors to stomach cancer (13) has generated new possibilities for its prevention. About 75% of the 870,000 annual noncardia gastric cancers in the world are attributable to *H. pylori* infection (14). Although *H. pylori* was not mentioned in epidemiological reviews of stomach cancer a few decades ago (15, 16), screening tests for the bacterium and treatment with antibiotics are now effective interventions for preventing stomach cancer (17). Epidemiological studies have also established salt consumption, smoking, and diets that are low in fruits and vegetables as risk factors for stomach cancer (16). In the United States, where *H. pylori* prevalence is lower than most other world regions (18, 19), these lifestyle and dietary factors together account for about 60% of stomach cancer deaths (20) (noting that different risk factors may be jointly responsible for some cases).

Although we can now leverage our knowledge about *H. pylori*, salt, smoking, and fresh

fruits and vegetables for preventing stomach cancer, the trends in stomach cancer also provide an example of how prevention may in fact precede and even help with risk factor identification. Adult death rates from stomach cancer are now close to 5 per 100,000 in Canada, the United States, and a few other western countries but reach 20 and 50 among Japanese women and men, respectively; half a century ago, they were as high as 150 to 200 among men in Japan, Chile, and Finland. The impressive declines in stomach cancer began before the epidemiological studies that identified these risk factors. Reductions in salt intake appear to have played an important early role in stomach cancer decline at least in Japan and Finland (15, 16, 21, 22). Stomach cancer prevention was also facilitated through improvements in hygiene, the living environment, and the uptake of refrigerators, which reduced the need to use salt for preserving food, improved the storage of fruits and vegetables, and may have also

reduced infection rates (15, 23)—creating an unintended success in prevention.

In an even more extreme example than stomach cancer, virtually all cervical cancers are due to infection with human papillomavirus (HPV) (14). Although sexual behavior and hygiene have traditionally been major determinants of geographical patterns and trends in cervical cancer, these cancers are preventable through early detection and treatment of precancerous lesions and, more recently, vaccination. However, access to regular cervical cancer screening, and especially vaccines, is lacking in low-income countries and in disadvantaged social groups (24), where the burden of cervical cancer is the highest. As a result, there is a 10-fold difference in cervical cancer incidence between countries in sub-Saharan Africa, where incidence is highest, and those in the Eastern Mediterranean and Western Europe, where it is lowest (25). Preventing cervical cancer in countries and communities with limited resources requires



**Fig. 2.** Trends in death rates from (A) lung cancer and (B) cardiovascular diseases in adults 30 years of age and older in selected countries with vital registration and medical certification of the underlying cause of death. Death rates are age-standardized to the WHO standard population for those aged 30 years and older and smoothed using a 5-year moving average. Source: WHO database of vital statistics, adjusted for completeness of death registration and for validity and comparability of cause-of-death assignment.

strengthening the primary care system, improving access to preventive care, and using the available interventions in such a way that they reach people who have fewer health system contacts (26).

In contrast to the above cancers that can be largely prevented through interventions and actions related to one or few dominant risk factors in most populations, other cancers have more diverse risk factors. For example, the risk factors for liver cancer and liver cirrhosis include infection with hepatitis B and C viruses (HBV and HCV); exposure to aflatoxin due to specific food-handling practices and storage conditions; alcohol use, especially binge drinking; and (for liver cancer but not liver cirrhosis) smoking. Each of these risk factors increases the risk of disease by a smaller amount compared with those discussed earlier for lung, stomach, and cervical cancers. As a result, the trends and geographical patterns of liver cancer and cirrhosis depend on the overall risk-factor profiles, and their population-based prevention should target locally relevant risk factors. HBV prevalence is highest in sub-Saharan Africa and in East Asia, where it is responsible for a large proportion of liver cancers; HCV's role is most important in sub-Saharan Africa, the Middle East, Central and East Asia, and Eastern and Southern Europe (14, 27, 28). Among the most effective means of preventing new HBV and HCV infections are reducing unnecessary medical injections, providing sterile syringes, and a safe blood supply in health care settings (29). Alcohol's role is largest in Eastern Europe (Box 1), especially among men, but it has a relatively small role in the Middle East and South Asia (30); that of tobacco is highest in Western countries, where people, especially men, have smoked for a long period.

### Modifiable Risk Factors and Cardiovascular Diseases: Prevention of Diseases with Diverse Risk Factors

Even more than liver cancer, CVDs have a large number of risk factors, each increasing disease risk by a relatively small amount. For example, smokers are two to three times as likely as those who have never smoked to die of CVDs, compared with 20 times for lung cancer. The diversity and the combinations of CVD risk factors across individuals and populations create more subtle variations in disease risks and rates than for the cancer risk factors described earlier. This in turn makes it more difficult to identify their independent roles in disease causation and prevention. Yet the evidence from studies of individuals and populations is by now equally compelling that reducing a moderate number of risk factors will have large benefits in CVD prevention. Further, given that the burdens of major CVDs like ischemic heart disease (IHD) and stroke are many times those of most cancers and other NCDs, reducing a prevalent risk factor like high blood pressure can prevent a very large number of disease cases or deaths, even if the

reduction in risk for each individual person is modest. The seminal work of Rose laid out the foundations of population-based prevention on the premise that "a large number of people at a small risk may give rise to more cases of disease than a small number who are at a high risk" (31).

The first line of evidence that CVDs can be prevented at the population level comes from their variations across countries and their long-term trends, which are as impressive as those of cancers. In the 1950s, age-standardized CVD death rates among adult men in Finland, Australia, and the United States were ~1200 per 100,000, compared with less than 600 in Greece and 700 in Norway (Fig. 2B)—a range that is much larger than that of lung cancer in

Fig. 2A. The range was narrower but still relatively large for women (Fig. 2B). Over the subsequent six decades, CVD mortality declined in most high-income countries—steadily and by about two-thirds in some of the countries that had started with the highest mortality. The decline began later, and was slower (including, perhaps, periods of increase), in some of the countries that had low starting mortality like Greece and Norway, and was further delayed in Central and Eastern European countries like Hungary. As a result, except in Central and Eastern Europe, the range of cardiovascular death rates in countries with long-term data is now narrower than it had been a few decades ago. These trends have also changed the ranking of countries

### Box 1. Hazardous alcohol use and cardiovascular diseases in Eastern Europe: A catastrophe in disease prevention.

Alcohol use is a risk factor for multiple NCDs, injuries, and even infectious diseases like tuberculosis. When consumed moderately and regularly, it has protective effects on CVDs and diabetes (96). On the other hand, irregular heavy (or binge) drinking reverses the protection against CVD and increases the risk of liver disease (97, 98). Hazardous alcohol use has created one of the most catastrophic public health phenomena of recent decades in Russia and some other former Soviet Republics. In these countries, hazardous alcohol use, including medicinal and industrial ethanol, is the leading cause of disease burden among all major risk factors, especially for men (99). Although a part of this burden is due to deaths from injuries in young adults, the majority is due to effects on NCDs, especially cardiovascular diseases (100), leading to the highest NCD death rates in the world (33). The rise in alcohol-related NCD mortality in Russia was so large that it led to a rise in total mortality (101), a situation that is rare outside of epidemics. Although the scale of the problem is unique in Eastern Europe, deprived and marginalized social groups in most places are affected by harmful alcohol use.

This failure of disease prevention has deep social and policy roots. Gorbachev-era policies had helped lower alcohol consumption, leading to lower mortality. Disintegration of the former Soviet Union was followed by a collapse of the social support and welfare systems and a rise in unemployment, deprivation, and stress. Alcohol control policies were also abandoned. Possibly the single most effective tool for NCD prevention in these countries and social groups is control of harmful alcohol consumption through social programs, taxes, and regulation.





in terms of their cardiovascular mortality. Finnish and Norwegian women now have nearly identical mortality, with the difference among men reduced to ~100 per 100,000 (compared with more than 800 in the 1950s). The decline in Australia, which started off with one of the highest mortality levels, has outpaced the United States and most other countries since the 1980s; as a result, Australia now has, together with France and Japan, some of the lowest levels of cardiovascular mortality ever recorded. There are less data on CVD trends in low- and middle-income countries. The available data nonetheless indicate that relatively soon after the decline in infectious diseases, CVD mortality also declines even in low- and middle-income countries (32–34).

What do these differences in CVD mortality level and trends, especially the success of countries like Australia and Finland, tell us about prevention? Faster emergency response times; use of medicines such as antiplatelet agents, angiotensin-converting enzyme inhibitors, beta blockers, and statins after heart attack or stroke; and medical advances such as angioplasty, defibrillation, and thrombolysis have improved the survival of people with a cardiovascular event (35). However, the contribution of postevent treatment to lowering the burden of cardiovascular diseases is less than 40 to 50% (36, 37); rather, the mortality decline is largely a result of lower disease occurrence, itself due to actions related to prevention.

In parallel to these improvements, our knowledge about cardiovascular risk factors has also advanced in studies of individuals and populations (38), providing the foundation for actions and interventions that can continue the past successes and replicate them in other populations. After clinical and early epidemiological research (39), studies with detailed measurement of risk factors and long follow-up, like the Framingham Heart Study, established elevated blood pressure and cholesterol, smoking, and excess body weight as some of the most important risk factors for CVD (40–42). These were followed by larger observational studies in Western and Asian populations that provided more details on the effects of these risk factors on CVDs (43–47); for blood pressure and cholesterol, there is also experimental evidence from randomized trials (48, 49). These studies showed that the benefits of lowering blood pressure, cholesterol, and body weight continue to very low levels—as low as 110 mmHg for systolic blood pressure (SBP), 3.8 mmol/L for serum total cholesterol (TC), and 21 kg/m<sup>2</sup> for body mass index (BMI) (20, 48). [For comparison, in 1950, systolic blood pressures as high as 175 mmHg were considered normal (50)]. Some researchers have even argued for abandoning the concept of hypertension and focusing on all feasible actions to lower blood pressure (51).

Reducing these risk factors in whole populations has contributed to past successes in CVD reduction in a number of countries (37). For ex-

ample, high-quality surveillance data show that the impressive declines in Finland are due to reductions in blood pressure, cholesterol, and smoking, despite the rising BMI levels (52). In 1980, Finnish adults had one of the highest blood pressure and cholesterol levels in the world: Mean SBP of adult Finnish men and women was 143 and 138 mmHg (53), respectively; their serum TC was above 6.1 mmol/L (54–56). Since then, SBP in Finland has declined by about 10 mmHg and TC by about 1 mmol/L (55, 56).

Yet there is more to be gained by further reducing these risk factors at the population level, especially in low- and middle-income countries. Blood pressure has declined in high-income countries but has increased in some other regions over the past few decades. As a result, blood pressure levels are currently highest in countries in Central and Eastern Europe and in parts of sub-Saharan Africa (55). Cholesterol is still highest in Western countries but has been increasing in East Asia (56). High blood pressure and cholesterol are each responsible for an estimated one-half of the global IHD burden, high BMI for about 20%, and tobacco smoking for 13% (57)—noting that the combined effect of these risk factors is much smaller than the sum of their individual effects because many people are exposed to multiple risks and because some of the effects of BMI on cardiovascular diseases are mediated through blood pressure and cholesterol. Similarly, high blood pressure is responsible for nearly two-thirds of stroke burden worldwide, with the other three risk factors each individually responsible for 12 to 18%. When overlaps are taken into account, these four risks together account for 70 to 80% of the burden of IHD and stroke (57). Importantly, the benefits of reducing these key CVD risks not only are very large but also can occur relatively fast and be fully realized within about 5 years, compared with about three decades for achieving the full benefits of smoking cessation on lung cancer and chronic obstructive pulmonary disease (58, 59). This means that actions to reduce key CVD risks can have immediate benefits for disease prevention and also contribute to reduced demand and cost of specialist treatment.

### Is It Feasible to Reduce Major Risk Factors and Prevent NCD?

Identifying risk factors is important but not sufficient for prevention. What is needed is evidence that risk factors can be reduced in whole populations and implementing policies and programs that can do so (60). Examples of such population-based reductions were provided above for some cancer risk factors.

The most basic evidence that it is feasible to prevent and reverse the rise of risk factors like blood pressure, cholesterol, and smoking comes from the differences in their levels and trends across populations, as stated earlier. More importantly, evidence has also accumulated that a

few feasible actions can achieve risk-factor reduction. Tobacco control interventions and policies have helped bring smoking prevalence below 20% in countries such as Australia and Canada (2). The most effective actions for lowering blood pressure and cholesterol, with evidence from individual- and population-based studies (22, 61–63), include lowering salt intake, replacing saturated fats with polyunsaturated fats, and clinical management of blood pressure and cholesterol with antihypertensives and statins through the primary-care system. Diets high in fruits and vegetables (64) and increased physical activity (65) also improve metabolic risk-factor profiles but need more systematic assessments of what combination of policies and actions can increase their uptake in the population, of the sort done in the Finnish cardiovascular risk-reduction experience (66).

In contrast with smoking, blood pressure, and cholesterol, reducing or even curbing the rise in overweight and obesity has proven particularly difficult. BMI has on average risen by as much as 2 to 2.5 kg/m<sup>2</sup> per decade and is now 30 kg/m<sup>2</sup> or higher in the Pacific islands and in some countries in the Middle East (67). Not only does high BMI increase the risk of cardiovascular diseases and some cancers, it is also the most important modifiable risk factor for glycemia and diabetes mellitus. Therefore, the worldwide rise in BMI has been accompanied by increasing diabetes mellitus in most countries (68), with more than one in four or five adults in some countries in the Pacific and in the Middle East now having diabetes (68). In tightly controlled studies of dietary change, moderate weight loss for up to 2 years has been observed, but evidence is lacking on the effectiveness of long-term and large-scale programs (69). Weight loss also appears to be more difficult than avoiding weight gain, perhaps due to specific physiological responses (70). A few controlled studies have successfully slowed down or even reversed a rise in blood glucose, and hence prevented diabetes, among people with impaired glucose tolerance that precedes diabetes, through improved diet and lifestyle and medicines (71, 72). However, the evidence on large-scale prevention and management at the population level is very limited (73). Put simply, we need to find ways that curb and then reverse the massive rise in weight gain if we are to prevent a pandemic of diabetes and to continue and replicate the CVD decline.

### The Way Forward in NCD Prevention

The 2011 United Nations High-Level Meeting created a window of opportunity for NCDs to receive global policy attention. Yet public health and health care systems in countries at all stages of economic development face the need to prioritize among numerous policies and programs related to prevention and treatment, to find financial and human resources to implement them, and to demonstrate that they improve people's health. Strengthening and supporting NCD pre-

**Table 1.** Effective approaches for large-scale NCD prevention.

Prevention mechanism	Action or policy	Evidence of successful implementation at scale	Prevention benefits
Eliminate or substantially reduce tobacco smoking	Comprehensive tobacco control, including taxes to increase prices; restricting availability and accessibility through regulation of sales; warnings; restricting advertising/marketing; public smoking bans (79)	Multiple high-income countries and some low- and middle-income countries	Multiple cancers; cardiovascular diseases; diabetes; chronic respiratory diseases; some other NCDs; respiratory infections and tuberculosis
Eliminate or substantially reduce harmful alcohol use	Comprehensive alcohol control, including taxes to increase prices; restricting availability and accessibility through regulation of production and sales; restricting advertising/marketing; enforcing drinking and driving laws (80, 81)	Some high-income countries and a few middle-income countries (82)	Multiple cancers; cardiovascular diseases*; liver cirrhosis and other gastrointestinal diseases; intentional and unintentional injuries; some infectious diseases
Reduce dietary salt intake to low levels	Taxes; regulation; well-designed public education; perhaps negotiated voluntary actions by food manufacturers (79, 83)	Finland, the United Kingdom, Japan, and a few other high-income countries (22, 62, 83); evidence lacking in low- and middle-income countries	Stomach cancer; blood pressure with benefits for cardiovascular disease and kidney disease
Eliminate manufactured trans fats	Ban partially hydrogenated oils	A few high-income countries (whole country or individual communities) (84, 85)	Cardiovascular diseases
Increase fresh fruits and vegetables in diet	Improving financial and physical access through price mechanisms (e.g., subsidies), agricultural policies, and possibly requiring availability in grocery stores; well-designed public education	Finland (66); some high-income countries but possibly due to broader changes in availability versus specific policies	Cardiovascular diseases; some cancers
Replace saturated fats with poly-unsaturated fats; replace processed carbohydrates with whole grains	Taxes/subsidies; regulation; labeling; perhaps negotiated voluntary actions by food manufacturers; well-designed public education	Finland, New Zealand, and a few other high-income countries for fat replacement (66, 86, 87)	Cardiovascular diseases; diabetes mellitus
Reduce overweight and obesity and increase physical activity	Design, implement, and evaluate actions and strategies for weight management/loss and for increasing physical activity at the population level (88, 89)	None	Cardiovascular diseases; diabetes mellitus; some cancers
Provide clean fuels for cooking and heating	Develop and deliver clean fuels for cooking and heating (90, 91)	Multiple middle-income countries, but possibly due to economic development versus specific policies	Chronic and infectious respiratory diseases; lung cancer; cataracts; possibly cardiovascular diseases
Eliminate or substantially reduce infections that are risk factors for, or predispose to, cancers and cardiovascular diseases	Vaccination for infections related to cancers, including HPV and HBV <sup>†</sup> ; treatment for the above plus HCV, <i>H. pylori</i> , schistosomiasis, and for bacterial infections like Chagas and Lyme disease and group A streptococcal tonsillitis and pharyngitis <sup>‡</sup>	Universal childhood HBV vaccination in some countries (92); relatively high coverage of cervical cancer screening in many middle- and high-income countries (24); successful schistosomiasis control in some developing countries (93); fewer examples, mainly in high-income countries, for other interventions	Cervical, liver, stomach, and bladder cancers; liver cirrhosis; atherosclerosis cardiomyopathies; rheumatic, valvular, and other heart disease; heart failure
Screen for and treat risk factors for NCDs in primary care	Implement an equitable and high-quality primary-care system (94); ensure availability of essential, and typically low-cost, medicines for NCD prevention and early-stage treatment	High-income countries with universal insurance; also implemented in low- and middle-income countries with focus on maternal and child health but has been extended to NCD management in a few countries (73)	Screening, early detection, and treatment of multiple NCDs and their risk factors
Ensure that all prevention strategies are designed to reach disadvantaged and marginalized communities and social groups.			

\*Effects of specific cardiovascular diseases depend on the patterns of alcohol consumption (regular moderate versus irregular heavy drinking). †Vaccines are not currently available for HCV and *H. pylori*. ‡Influenza vaccination among people with coronary heart disease and heart failure is also a secondary prevention that reduces their risk of dying (95).



vention requires a broader notion of preventability than detecting causes that increase disease risk in individuals—one that incorporates the potential for large change in whole populations. Our knowledge from studying NCDs in individuals and populations shows that there are ways to achieve the large-scale disease prevention potential, e.g., in whole countries and communities (Table 1). The policies and actions to change NCD risk factors of the kinds specified here need political support and policy and administrative institutions that can initiate, periodically evaluate, and as needed redirect them. However, once implemented, unlike treatment (which often deals with a single disease), most of these actions can prevent the occurrence of multiple NCDs and can therefore have very large benefits for the health of populations.

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PERSPECTIVE

# Preventing Alzheimer's Disease

Dennis J. Selkoe

Despite intensive laboratory and clinical research over three decades, an effective treatment to delay the onset and progression of Alzheimer's disease is not at hand. Recent clinical trial failures suggest that we must treat the disease earlier than in its mild to moderate stages, and major progress in validating presymptomatic biomarkers now makes secondary prevention trials possible. We will learn more about the natural history of the disease and any partial therapeutic responses from detailed analyses of recent trial results. This process will likely position the field for success, but only with much greater investment in all aspects of Alzheimer research and with careful design of future trials.

Few diagnoses in modern medicine evoke deeper apprehension in patient and family than Alzheimer's disease (AD). The implications of having cardiovascular disease, cancer, or metabolic disease are ominous, but surveys suggest that people particularly fear developing AD. This is so because Alzheimer's robs us of our most human qualities—reasoning, memory, abstraction, language, emotional control—and because a disease-modifying treatment remains beyond reach. This enormously common neurodegenerative disorder affects more than 5 million Americans and well over 35 million worldwide, numbers expected to grow dramatically as the population ages and competing causes of death in late life continue to recede (1). The projected rate of rise is even greater in the developing world than in the high-income countries (2) (Fig. 1).

As with other slowly progressive diseases, preventing AD depends on understanding early steps in its pathogenesis. A worldwide research effort during the past quarter century has yielded an increasingly detailed picture of the cytopathological, biochemical, and genetic underpinnings of the disease, including in its presymptomatic phase, and the parallel development of biomarker and neuroimaging modalities [reviewed in (3, 4)]. The classical lesions that Alois Alzheimer called attention to a century ago—extracellular amyloid plaques and intraneuronal neurofibrillary tangles—were shown in the mid-1980s to be composed, respectively, of the 42-amino acid  $\beta$ -amyloid protein (A $\beta$ 42) and the microtubule-associated protein tau. By the mid-1990s, decreased A $\beta$ 42 levels and increased tau levels in cerebrospinal fluid (CSF) were associated with a clinical diagnosis of AD (5). Soon, lowered CSF A $\beta$ 42 levels were documented in older people who appeared to have very early AD (sometimes referred to as mild cognitive impairment—amnesic type) or were still cognitively normal (6), with the rise in CSF tau levels generally following the A $\beta$ 42 decline (4). In the realm of neuroimaging, progressive hippocampal

and cortical atrophy were measured with increasing precision before and during the clinical phase of AD, and this brain shrinkage was found to be accompanied by decreased cerebral metabolism on fluorodeoxyglucose positron emission tomography (FDG-PET). By 2004, the synthesis of a blood brain barrier penetrant, radiolabeled analog of the amyloid-binding dye thioflavin T [Pittsburgh compound B (PiB)], enabled researchers to image fibrillar amyloid deposits in vivo by PET (7). Taken together, these quantifiable markers of the evolving disease process in living human patients, now widely replicated in multiple studies (8), provide a critical resource for validating preventative and therapeutic agents in AD.

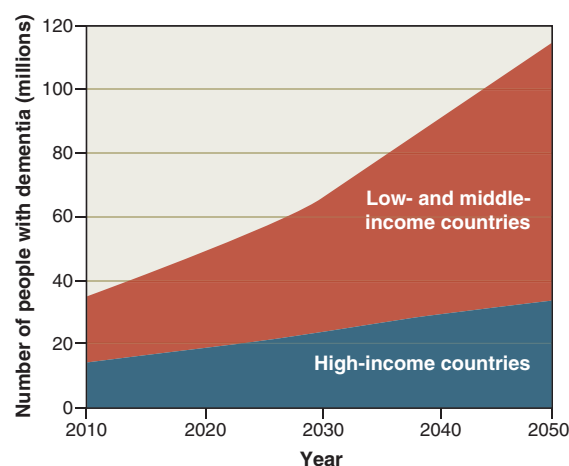
## Pinpointing a Predisposition to AD

A small fraction (<1%) of all AD cases arises during middle age because of inherited missense mutations in one of three genes: *APP*, *PSEN1*, or *PSEN2*. The *APP* gene codes for the 695-amino-acid-long  $\beta$ -amyloid precursor protein (APP), which has salutary functions during brain development and in various biological processes in adulthood (9). All AD-causing mutations in APP alter amino acids within or immediately flanking

its small ~42-residue A $\beta$  region. The *PSEN1* and *PSEN2* genes code for two homologous (and redundant) intramembrane aspartyl proteases, presenilin 1 and presenilin 2 (10, 11). Therefore, all of these AD-causing mutations directly affect the biochemical reaction that generates A $\beta$ 42 and related peptides throughout life, by altering the substrate (APP) or the protease that cleaves this substrate (i.e., presenilin, the catalytic component of  $\gamma$ -secretase). The full penetrance of the mutations for early-onset, autosomal dominant AD and the fact that they result in a neuropathological, biochemical, and clinical phenotype largely indistinguishable from typical, late-onset ("sporadic") AD provide strong genetic evidence for the amyloid or A $\beta$  hypothesis, which posits that AD arises in substantial part from a chronic imbalance between A $\beta$  production and A $\beta$  clearance in the brain. Numerous families carrying *APP*, *PSEN1*, or *PSEN2* mutations have been studied collectively to determine the time course of fluid biomarker changes, neuroimaging changes, and clinical changes before the expected onset of AD symptoms, which is based on the age of symptom onset in a parent with the same mutation.

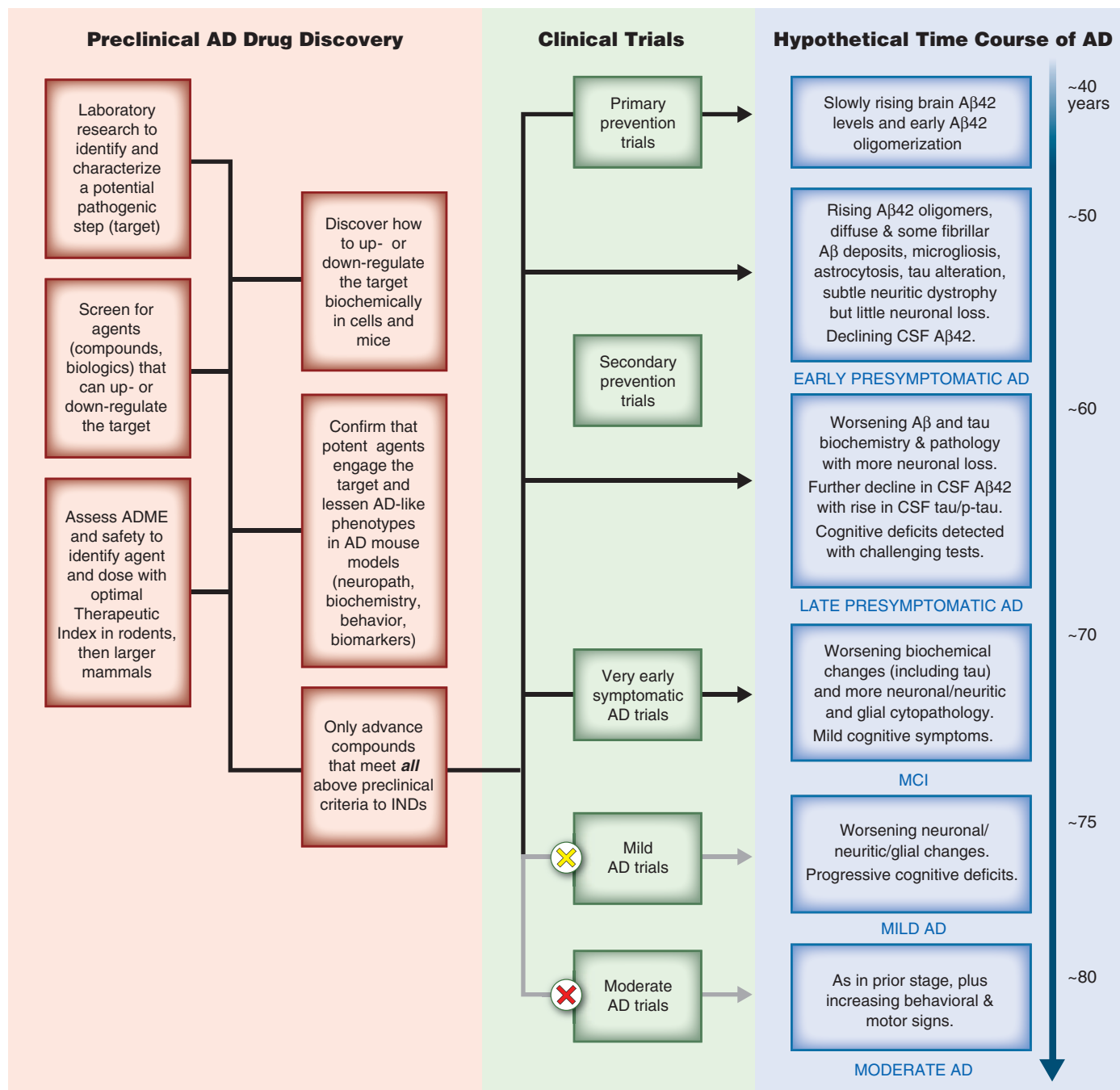
Initial analyses of a familial AD cohort [the Dominantly Inherited Alzheimer Network (DIAN)] suggest that A $\beta$ 42 levels in CSF begin to decline as early as 25 years before expected symptom onset (12). This is followed by the appearance of fibrillar amyloid deposits in the brain (as detected by PiB-PET), increased levels of tau in CSF, and progressive brain atrophy roughly 15 years before expected symptom onset (12). Cerebral hypometabolism and subtly impaired episodic verbal memory seem to begin some 10 years or so before expected symptoms (12). If this time course is generally similar to that of sporadic AD, and there is evidence from cross-sectional studies that it may be (4), then humans destined to develop AD have detectable biochemical and histopathological abnormalities two decades or more before overt clinical symptoms (Fig. 2).

Two key lessons that emerge from such studies of presymptomatic AD are that (i) profound brain alterations occur well before the dementia can be diagnosed (13) and (ii) therapeutic interventions directed only at the mild-to-moderate clinical stage may be too late to ameliorate symptoms. The latter conclusion is supported by recent phase 3 trials of certain A $\beta$ -clearing monoclonal antibodies (e.g., bapineuzumab) that apparently failed to significantly slow cognitive and functional decline over 18 months, even though such antibodies are capable of preventing further rises in amyloid burden (14, 15) and lowering CSF phospho-tau levels, a key biomarker of AD-type neuronal degeneration (16).



**Fig. 1.** Projected increases in the numbers of people with dementia in high-income countries and in low- and middle-income countries. [Figure reproduced, with permission, from (2)]





**Fig. 2.** Aligning potential disease-modifying agents for AD with the course of the disease. Red boxes indicate the sequence of steps in the discovery of compounds or biologics as investigational new drugs (INDs) for AD. Blue boxes, speculative stages in the long presymptomatic and symptomatic phases of AD in a hypothetical individual who undergoes A $\beta$  buildup for one of

several possible reasons (e.g., a presenilin or APP mutation, ApoE4 inheritance, increased  $\beta$ -secretase activity, etc.) and develops very early symptoms by around age 70. Green boxes, clinical trial categories dependent on the stage of AD. Red X, trials in moderate AD not recommended. Yellow X, trials in mild AD recommended with caution. [Figure adapted from (35)]

### Moving Toward Prevention Trials

Although antibodies against A $\beta$  (anti-A $\beta$ ) that enhance clearance of the peptide and other A $\beta$ -lowering agents, such as inhibitors of the  $\beta$ - and  $\gamma$ -secretase enzymes that generate A $\beta$ , may still be shown to provide benefit in mildly symptomatic AD, the AD field has moved toward a consensus

that secondary prevention (diagnosing and treating the disease before overt symptoms) is more likely to slow the pathogenic process (17–19) (Fig. 2). Such trials might be designed in the following way. Presymptomatic participants who bear deterministic mutations in *APP*, *PSEN1*, or *PSEN2* (i.e., autosomal dominant AD) could undergo treatment

with an A $\beta$ -lowering or -neutralizing agent beginning 2 to 5 years or more before the expected age of onset of frank symptoms. In such trials, enrollees would have low CSF A $\beta$ 42 levels as well as brain amyloid deposits (detected by PET imaging) that exceed normal thresholds, ensuring that they can potentially respond to an anti-A $\beta$  treatment.

Participants who do not yet have elevated CSF levels of tau or phosphorylated tau at trial entry could be compared with participants who already have these markers of tangle-associated neurodegeneration. Regarding outcomes, the treatment's capacity to delay the aforementioned biomarker changes [amyloid-PET and FDG-PET abnormalities; cerebral atrophy by volumetric magnetic resonance imaging (MRI); rising CSF levels of tau and phosphorylated tau] could be assessed yearly to ascertain the earliest point of deflection from the pathogenic trajectory of placebo-treated mutation carriers. Salutary movements of biomarkers would be expected to be seen first, but these might be accompanied by less decline on challenging cognitive tests that are sensitive to the cardinal manifestations of very early clinical AD (e.g., episodic memory loss and word learning and retrieval deficits) rather than more global cognitive tests [e.g., Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog), MiniMental State Examination (MMSE)] that have less sensitivity to detect early progression. Combined assessment of AD biomarker status and episodic memory should indicate whether the agent in question can slow disease progression over a 3- to 5-year interval. An academic consortium sponsored in part by the U.S. National Institutes of Health (NIH) and the biotechnology company Genentech has recently been approved to carry out such a secondary prevention trial with an anti-A $\beta$  (crenezumab) in ~300 presymptomatic members of a large Colombian pedigree with the presenilin 1 Glu<sup>280</sup>→Ala<sup>280</sup> missense mutation and a smaller number of presymptomatic American participants from the DIAN cohort who carry other presenilin mutations (20). Another secondary prevention trial in autosomal dominant AD has been proposed to the NIH by the Alzheimer's Association and a consortium of DIAN sites and certain pharmaceutical companies (21).

In addition to conducting prevention trials in presymptomatic participants with rare, dominantly inherited AD, it is important to initiate similar studies as soon as possible in presymptomatic participants with common, late-onset AD (so-called sporadic AD). Here, it may be useful to divide participants by their apolipoprotein E genotypes, because the rate of AD pathogenesis and degree of  $\beta$ -amyloid burden are generally greater in patients with the E4 allele of this gene compared with those with just E3 or E2 alleles, and side effect profiles may differ. For example, phase 2 trials of bapineuzumab, a humanized monoclonal antibody to A $\beta$ , indicated that ApoE4-positive participants were significantly more likely to develop an MRI change designated ARIA-E (amyloid-related imaging abnormality—edema or effusion; formerly called vasogenic edema) than were ApoE4-negative participants, presumably related to the heavier burden of microvascular amyloid in the former (22). Although only a small minority of participants treated with passive A $\beta$  immunotherapy show ARIA-E on MRI, and most of these

experience no overt symptoms, subtle effects of this process on their mental status are possible and could partially mitigate any cognitive benefits of lowering and neutralizing A $\beta$ . For some types of experimental anti-A $\beta$  therapeutics, for example, the  $\beta$ - or  $\gamma$ -secretase inhibitors now under active development, prospective separation of trial participants by ApoE4 genotype may not be necessary, and stratification of the outcomes by genotype can be done at trial's end. A secondary prevention trial of an anti-A $\beta$  in sporadic AD subjects (the A4 trial) is currently in advanced stages of planning by a consortium led by the Alzheimer's Disease Cooperative Study group funded by NIH (23).

### Markers Before Mentation

As we move toward more optimal times for disease modification (i.e., very early symptomatic AD and secondary prevention before symptoms), the importance of obtaining CSF samples by lumbar puncture on as many trial participants as possible cannot be overstated. CSF provides a virtually ideal indicator of the biochemistry of the fundamental pathogenic processes of AD in vivo and in real time. The fact that multiple lengths of A $\beta$  peptides (especially A $\beta$ 42, 40, and 38) and total and phosphorylated tau proteins can be quantified in small samples of CSF allows one to acquire simultaneous information about a putative pathogenic agent (A $\beta$ ) and a critical neuronal response molecule in AD (tau). Preclinical research shows that the expression of tau is necessary for A $\beta$  peptides [including soluble oligomers that may contribute prominently to neuronal dysfunction (24)] to induce neuritic dystrophy and cytoskeletal collapse in cultured neurons (25) and behavioral deficits in APP transgenic mice (26). In the future, additional CSF analytes (e.g., other neuronal proteins, monocyte- and microglia-derived cytokines, certain lipids, metal ions, etc.) could provide a more sophisticated picture of the AD biochemical phenotype in vivo and also be used as biomarkers of progression.

The notion that lumbar puncture is uncomfortable and generally unacceptable to patients is badly out of date. Some investigators in Europe have been collecting CSF samples from participants with symptomatic and presymptomatic AD for many years, and clinicians in the United States need to catch up. The Alzheimer Disease Neuroimaging Initiative (ADNI; sponsored by the NIH and numerous biopharmaceutical companies) and some individual AD research centers have documented how feasible and informative routine CSF analysis can be [e.g., (27, 28)]. Patients and their families are usually amenable to lumbar puncture once they are informed of how important it is for accurate diagnosis and for assessment of trial outcomes, and the procedure can be quickly and safely performed in an outpatient setting (29). Ideally, each academic center or practice participating in AD trials should designate one or two highly

experienced physicians to perform all the lumbar punctures, as is done for other minimally invasive diagnostic procedures such as arthroscopy. Although the advent of PET amyloid imaging is a great boon to the presymptomatic diagnosis of AD, a low CSF level of A $\beta$ 42 is an equally if not more sensitive biomarker indicating that cerebral A $\beta$  deposition is underway (28), and this information can be acquired at less expense than a PET scan.

Such considerations underscore the reality that we cannot validate efficacious disease-modifying agents in AD without strong reliance on biomarkers. The AD field often discusses the compelling example of blood lipid profiling in coronary artery disease (CAD), which led to regulatory approval of the first statin years before these low-density lipoprotein (LDL)-cholesterol-lowering drugs were unequivocally proven to prevent heart attacks (30). This achievement occurred because elevated cholesterol levels and abnormal lipoprotein profiles in blood had been tightly linked to the risk of progressive CAD by many epidemiological and mechanistic studies (31). The AD field is now approaching an analogous benchmark, with numerous CSF and neuroimaging studies consistently validating early changes in CSF A $\beta$ 42 and tau levels and cerebral amyloid deposition as indicative of a high risk of developing disease (8). It took years of clinical use of statins to be certain that therapeutically lowering LDL-cholesterol helped prevent morbidity and mortality in CAD (i.e., prove the cholesterol hypothesis) (30), and yet statin treatment was approved and rapidly expanded before that. Because neuropathological, genetic, mechanistic, biomarker, and even therapeutic research all support an early pathogenic role of A $\beta$ 42, the AD field needs to follow suit.

Although sensitive memory tests should always accompany biomarker assays in secondary prevention trials, rigorous and statistically significant outcomes on brain and CSF A $\beta$  and tau should be considered for regulatory approval as long as an amyloid-lowering agent is deemed safe. Only with more widespread and prolonged use of a well-tolerated agent that hits AD biomarker endpoints can we ultimately determine whether the agent is efficacious on quality-of-life outcomes. Surveys indicate that many patients and their families are willing to undergo experimental testing of preventatives or early treatments, given the current absence of an approved disease-modifying therapeutic for this terrible, fatal disease. Approval of a safe agent that was designed on the basis of our current best understanding of AD mechanism should be considered on biomarker grounds alone, as has sometimes occurred in other chronic, life-threatening diseases.

### Beyond A $\beta$

Why have agents targeting A $\beta$  received so much therapeutic attention in AD? The principal reason is the wealth of evidence from many independent investigators worldwide supporting an early



role for A $\beta$  dyshomeostasis in AD pathogenesis. Nonetheless, there remain appropriate concerns about A $\beta$  as a cause and as a worthy therapeutic target in AD. Some of the considerable controversy that has swirled around this topic may represent misunderstandings of data and goals. I will describe just two key examples. First, there remains debate about whether A $\beta$  accumulation is a cause or an effect of AD. Almost certainly, the answer is both. When APP or presenilins are mutant, A $\beta$  overproduction appears to be the earliest identifiable molecular event associated with the development of AD. But in the vast majority of cases, an imbalance between A $\beta$  production and clearance, which occurs in 100% of patients as we define AD, is caused by other upstream events, most currently unknown. One very important known cause is inheritance of one or two  $\epsilon$ 4 alleles of the apolipoprotein E gene (32). Such cases were once part of the broad swath of sporadic AD, but we now recognize ApoE4 as the single greatest risk factor for the disease besides age. Compelling evidence indicates that the ApoE4 protein decreases cellular clearance of A $\beta$  and enhances the stability of extracellular A $\beta$  aggregates in brain (33), but evidence for additional, non-A $\beta$ -mediated effects, including on tau, is also accruing (34). Even though A $\beta$  cannot be said to be solely causative in ApoE4 carriers (who may number at least half of AD cases), an agent that chronically reduces A $\beta$  production (e.g., a  $\beta$ -secretase inhibitor or a  $\gamma$ -secretase modulator) or enhances its clearance (e.g., a passively administered A $\beta$  antibody or an active A $\beta$  vaccine) should be efficacious. In short, A $\beta$  is both cause and effect in AD.

A second misunderstanding is the notion that therapies lowering A $\beta$  could be dangerous because they would decrease the peptide's normal functions. Even years before symptomatic AD, brain levels of A $\beta$  are very substantially increased, and no currently contemplated therapeutic would be expected to reduce them to subphysiological levels, just as therapeutic statin doses do not cause serum cholesterol to fall to dangerously low levels. Whether the A $\beta$  fragment of APP acquired a biologically important function during evolution distinct from those of other proteolytic fragments of the precursor is under intensive study. For example, a recent report found that A $\beta$ 40 and A $\beta$ 42 peptides can favorably modify peripheral lymphoid and myeloid cell function to mitigate against experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (35). But lowering the excessive brain levels of soluble A $\beta$  peptides in AD to subphysiological levels is not contemplated and would be difficult to achieve.

These and other specific controversies surrounding the A $\beta$  hypothesis have been discussed (36) and are widely viewed as not precluding further human research on A $\beta$ -lowering strategies. At the same time, it is crucial to expend more preclinical and clinical research effort on non-A $\beta$  strategies—for example, lowering excess levels

of tau proteins or down-regulating inflammatory cells both centrally and peripherally. Unfortunately, these approaches are well behind therapeutic development in the A $\beta$  arena. We must substantially increase research on these alternative targets while also accelerating our judicious testing of A $\beta$ -directed agents in presymptomatic or very early AD. Current research funding for AD is not nearly sufficient; it still represents a modest portion of the dollars our field needs to study AD comprehensively, and it is a very small fraction (<1%) of the enormous costs our society bears each year to provide care to AD patients.

### Reducing AD Risk Without Drugs

Our understanding of environmental factors that modulate one's risk of developing AD lags behind our knowledge of the contributing genetic factors. Nonetheless, this important topic has been receiving increasing scrutiny. Lifestyle factors that may reduce the risk of dementia in general and AD in particular include physical exercise, cognitive activity and educational attainment, and social engagement. The protective effect of aerobic exercise (37, 38) may derive from enhanced cardiovascular fitness and cerebrovascular health but possibly also from modifying the biology of AD, for example, by lowering A $\beta$  accumulation or decreasing neurotrophic dystrophy. Lifelong intellectual activity and higher educational levels have been found to lessen the risk of developing AD or to ameliorate its course (39, 40). In mouse models, environmental enrichment, including repeated exposure to novelty, has been shown to decrease amyloid burden and attendant neuroanatomical and behavioral deficits (41, 42). Strong social engagement and lack of isolation in the elderly may contribute to a lower likelihood of developing dementia, including AD, or a slower progression of symptoms (43, 44). Our current state of knowledge suggests that changes in lifestyle are unlikely to be sufficient to stave off the development of AD, particularly if these changes are instituted close to the time of clinical onset. However, it is possible that exposure to beneficial lifestyle factors over many years could delay the onset and progression of the disease. This research area merits rigorous investigation, given the ongoing challenges of validating a safe disease-modifying therapeutic and the high costs that will be incurred with its chronic administration.

### Success from Failure

Attempts to treat complex, chronic diseases such as AIDS or certain forms of cancer have been marked by major failures before tangible success emerged. One hopes that this will be the case in AD. The appropriately intensive effort to test potential AD therapeutics earlier and in more carefully designed trials is likely to lead to rigorous scientific evidence of disease modification before long. This evidence may arise principally from biomarker data, although it will likely be accompanied by positive trends on cognitive tests. De-

spite being enormously disappointing to patients, families, and physicians, the field's recent clinical trial failures will provide a great deal of concrete information about what may work partially, what does not, and where to go next. As a society, we must invest much more and invest more wisely. We must broaden our therapeutic vision beyond A $\beta$  and also move quickly to trials in very early symptomatic as well as presymptomatic participants. As soon as we see independently replicated evidence of relevant biomarker changes and some cognitive benefit, we should begin to consider even earlier ("primary") prevention trials for middle-aged participants bearing ApoE4 alleles. Our patients and their families should remind us of Churchill's exhortation: "...never, never, ever quit!"

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## PERSPECTIVE

# Changing Human Behavior to Prevent Disease: The Importance of Targeting Automatic Processes

Theresa M. Marteau,<sup>1\*</sup> Gareth J. Hollands,<sup>1</sup> Paul C. Fletcher<sup>2</sup>

Much of the global burden of disease is associated with behaviors—overeating, smoking, excessive alcohol consumption, and physical inactivity—that people recognize as health-harming and yet continue to engage in, even when undesired consequences emerge. To date, interventions aimed at changing such behaviors have largely encouraged people to reflect on their behaviors. These approaches are often ineffectual, which is in keeping with the observation that much human behavior is automatic, cued by environmental stimuli, resulting in actions that are largely unaccompanied by conscious reflection. We propose that interventions targeting these automatic bases of behaviors may be more effective. We discuss specific interventions and suggest ways to determine whether and how interventions that target automatic processes can enhance global efforts to prevent disease.

At the 65th World Health Assembly held in Geneva in May 2012, health ministers pledged a 25% cut in premature deaths from the four most prevalent noncommunicable diseases—diabetes, cardiovascular disease, lung disease, and cancer—by 2025. Achieving this will require sizeable shifts in the population distribution of consumption of calories, tobacco, and alcohol, as well as increased levels of physical activity and fruit and vegetable consumption. But how might such changes in behavior be achieved? Hitherto, nonregulatory approaches to changing behaviors across individuals and populations have focused largely on using information to persuade people of the risks they face and the potential benefits of change, through clinics or public health campaigns, such as those aimed at increasing the consumption of fruit and vegetables or at reducing the consumption of alcohol. More recent variants of this approach include personalizing risk messages using the results of a wide array of biomarker tests, including blood glucose levels indicating an increased risk of diabetes and gene variants indicating an increased risk of heart disease. These approaches have had either modest or no effects on health-harming behaviors (1, 2).

We propose that the potential for information-based interventions is fundamentally limited,

given that it is based on a view of human behavior that is at odds with psychological and neuroscientific evidence that much human behavior is not actually driven by deliberation upon the consequences of actions, but is automatic, cued by stimuli in the environment, resulting in actions unaccompanied by conscious reflection (3).

## Flexibility Versus Efficiency: Understanding the Balance in Human Behavior

Throughout our day, we shift between two broad categories of behavior (4, 5). On the one hand, we may act in a reflective manner, directing ourselves toward particular goals, aware of our motivations and actions and able to halt or modify them should the need arise. In other instances, we act without reflection, responding to our surroundings in complex ways while our thoughts may be far removed. Each of these types of behavior has its advantages and disadvantages. The former is goal-directed, flexible, and rational insofar as it is motivated by explicit beliefs and desires. But it is also slow, cumbersome, and metabolically costly, absorbing our attention and preventing other processing. It is especially inefficient when it comes to routine situations: Why would one wish to deliberate over each stage of a familiar route home? The latter behaviors, in their automaticity, have the advantage of capitalizing on the routine and the predictable, freeing us to devote our cognitive capacity to other matters while nevertheless engaging in complex and fruitful actions. However, in becoming divorced from awareness and reflection, these automatic behav-

iors lose flexibility and may become out of touch with conscious desires, proceeding even when the consequences are unwanted. Thus, we may find ourselves taking the well-travelled route home when the original intention had been to call elsewhere.

Although it is usual to draw a complete distinction between these two broad categories of behavior, in fact they overlap and interact, with any given behavior consisting of a complex mixture of the two. Ideally, they would, and often do, complement each other, but they may also come into conflict. This is particularly so in the case of health-related behaviors, for which people often have competing goals (such as a pleasure goal of enjoying a cake versus a health goal of reducing weight). It is perhaps most useful to think of them in terms of a balance, with certain factors promoting the more reflective and others the more automatic behaviors. Thus, for example, engaging in a task that absorbs attention may shift us toward more automatic behavior. This is illustrated in an experiment showing that having to remember a long string of numbers made people more likely to select chocolate cake than fruit salad when presented with a forced choice in the middle of the experiment (6). Stress too can shift us from being rational and goal-directed to more automatic, responding to external stimuli and persisting in actions that are not ultimately helpful.

The above distinction is very relevant to established experimental work on habits, which are actions that occur in response to stimuli without necessarily bringing to mind the goal of that action. Habits are contrasted with goal-directed behavior and form one class of automatic behavior. They become established by repetition and routine, their emergence being marked by measurable changes in brain circuits (7). Although habits constitute an important class of automatic behavior, it is important to note that not all automatic behavior is habitual. For example, viewing a beer advertisement on television may result in the viewer going to the fridge for a beer without awareness of the

**“Ninety-nine hundredths or, possibly, nine hundred and ninety-nine thousandths of our activity is purely automatic and habitual, from our rising in the morning to our lying down each night.”**

William James (1899)

<sup>1</sup>Behaviour and Health Research Unit, Institute of Public Health, University of Cambridge, Cambridge CB2 0SR, UK. <sup>2</sup>Department of Psychiatry, University of Cambridge, Cambridge CB2 0SR, UK.

\*To whom correspondence should be addressed. E-mail: theresa.marteau@medschl.cam.ac.uk



link between the ad and her behavior (i.e., it is a behavior cued when the environmental cue elicits the goal or desire), but this need not be a habitual response to watching television. Additionally, automatic behavior can be goal-directed (8–10). Finally, although automatic behaviors are generally considered to occur without awareness, automaticity is best considered as a continuum, with some automatic behaviors cued and enacted entirely outside of awareness (such as the mimicry of non-verbal behavior in social interactions), whereas for others, the cue and the ensuing behavior may be noticed while the causal link between the two occurs outside awareness [such as occurs in the priming effects on consumption of the advertising of food and alcohol (11, 12)].

The key point here is that environmental cues can elicit both habitual actions in the absence of a conscious desire (the hand that dips into the open biscuit tin), or they can automatically, perhaps unconsciously, bring to mind a desire. In both cases, the behavior that emerges can be considered automatic and unlikely to be susceptible to modifications aimed at rational, reflective thought. This account of human behavior helps explain why health-harming behaviors persist in the population and are so resistant to change. We argue that the time is right to examine whether interventions that target these automatic, unreflective processes can change behavior on a scale that will contribute to realizing the World Health Assembly's ambition.

### Changing Human Behavior

Despite the work in brain and behavioral sciences demonstrating the dominance of automatic processes in guiding action, most interventions aimed at changing health-related behavior target reflective processes. At their simplest, these interventions entail providing information in an attempt to persuade people to change their behavior in, for example, mass media campaigns designed to increase the consumption of fruit and vegetables. At their more complex, they aim to impart skills to increase individuals' self-regulatory capacity to engage in healthier behavior, as found in stop smoking services and weight loss programs. Although the latter type of intervention can achieve sustained change in, for example, diet, physical activity, and smoking (13), their potential to change behavior on the scale needed to halt and reverse the rise in noncommunicable diseases is limited, because

only a minority of those with the unhealthy behavior engage in such programs and, for those that do engage, the effect sizes achieved are modest (2). Such effect sizes are smallest for routine learned behaviors. This accords with observations that high levels of training and repetition in experimental animals produce behaviors that are persistent and insensitive to changed or devalued outcomes (14). The very highly trained rat will continue to press a

consider making the elevator a less appealing option by increasing the effort required to use it. For example, slowing down the speed at which elevator doors close, thus increasing the journey time, increases stair use (16). Similarly, altering the effort required to reach foods in a cafeteria salad bar (Fig. 1) by manipulating their proximity by only around 10 inches, can increase the selection of easier-to-reach food options (17). Reducing the proximity and density of retail outlets for alcohol, tobacco, and junk food can also reduce purchasing and consumption (18).

*Availability of options within environments.* The availability of an option within a given environment increases the ease with which it can be used and thereby the likelihood of this. For example, commuters used the stairs nearly twice as much when there was only one ascending escalator available as an alternative to taking the stairs, as when there were two escalators (19). Increasing the number of healthier food and drink options in vending machines has also been shown to increase the likelihood that healthier choices will be selected (20).

*Product design.* The design of a product shapes our behavior in relation to it. Over time, many products central to health-related behavior have altered in ways that result in less energy expenditure and greater consumption. For example, 150 years ago, clerks worked at standing desks. The provision of modern equivalents in schools can result in pupils expending more calories (21). The size and shape of tableware, such as plates and drinking glasses, have also changed over the centuries, with both properties influencing eating and drinking behavior. For example, taller glasses resulted in less being poured and drunk than shorter, wider-bottomed glasses, although study participants perceived the opposite to be true (22).

*(ii) Targeting automatic associative processes.* We describe examples of interventions that deliberately target automatic processes to activate, inhibit, or alter existing associations or create new ones, so that individuals behave differently in reaction to environmental cues.

*Activating or inhibiting existing associations.* Priming is one mechanism to influence behavior outside awareness. It involves presenting a stimulus that activates or inhibits an associated mental representation (a concept, action, or goal). This alters the threshold for action and the likelihood



**Fig. 1.** Altering the effort required to reach foods by manipulating their proximity can increase the selection of easier-to-reach food options.

lever for a drink even when that drink has been made bitter. It accords too with our everyday experience that highly routine behaviors, including what and when we eat, are difficult to change.

There are many such interventions that probably require little, if any, conscious engagement or target automatic processes to change health-related behaviors, and that could be implemented at the population level. We outline below some examples from laboratory and field experiments. These fall into two broad but overlapping categories: (i) those that alter a person's environment and (ii) those that aim to target automatic processes (and thereby how an individual responds to environmental cues).

*(i) Altering environments to constrain behavior.* Reflecting Tolman's law of least effort, which was based on observing rats taking the shortest path in a maze, altering the properties of objects or their space within physical environments can constrain or shape responses to make the least effortful course the most likely, without a need to prompt conscious deliberation (15).

*Ease of effort.* Taking the elevator rather than the stairs typically requires less effort on the part of the individual. To encourage the more physically active behavior of using the stairs, we can

of its expression given subsequent exposure to a relevant stimulus. For example, priming the concept of old age (by exposing participants to words such as “wrinkles” and “gray”) led to participants walking more slowly when leaving the experiment (23). Priming effects have been demonstrated across a range of responses and stimuli, including the presentation of alcohol and snack food in advertisements. For example, children watching cartoons interspersed with food advertisements ate 45% more of the snacks made available to them (which did not appear in the advertisements) than children watching cartoons interspersed with advertisements for nonfood items. Adult consumption of snacks was also affected (11). Similarly, adults watching film clips that included scenes in which alcohol featured prominently, interspersed with advertisements for alcoholic beverages, selected more alcoholic beverages to drink afterward than did those exposed to either of these alone. Those exposed to film clips and advertisements that did not feature alcohol selected the fewest alcoholic beverages (12). In accordance with such findings, restricting marketing is considered one of the more potent interventions in reducing tobacco use, alcohol consumption, overweight, and obesity, particularly in children (24). The potential of using priming interventions to reduce consumption is promising (25) but little studied thus far.

*Altering existing or creating new associations.* Humans are generally predisposed to approach positive stimuli (those we anticipate as being rewarding) and avoid negative stimuli (those we anticipate as being unrewarding or even punishing). Products are frequently packaged with a variety of positive and negative associations, generating competing behavioral impulses. Increasing approaches toward healthier behaviors and products and the avoidance of less-healthy options involves increasing positive associations with the former and negative associations with the latter. So, for example, using fun terms to describe healthier foods increases the chances that children will eat them, as does putting cartoon characters on vegetables (26), whereas removing branded packaging of junk food and tobacco reduces the attractiveness of the products (27, 28).

The interventions described above may have additional benefits: Their delivery (for example, via a change in the physical environment) does not usually require complex systems or direct contact with people, thus allowing increased efficiency and decreased costs as compared with individually delivered interventions. They also have the potential to reduce health inequalities, because they do not rely on the communication and comprehension of complex information about health. The impact of interventions that involve providing persuasive information depends on recipients’ literacy, numeracy, and cognitive control, which are generally poorer in those who are more deprived (29–31). In contrast, changes made to the physical environment largely bypass these processes, having

the potential to shape behavior for all individuals who are exposed to that environment.

### Future Directions

The interventions we have described are, in principle, scalable to the population level, although uncertainty remains about whether they will be effective in changing behavior in populations living in complex environments and at a level needed to reduce the global burden of chronic disease. Several steps are needed to test this. First, we must identify which stimuli in which environments are most likely to achieve sustained healthy behaviors. For example, although the quantity and quality of space devoted to unhealthy and healthy products in a grocery store, as well as their packaging and marketing in and out of the store, will influence the relative healthfulness of the food bought, experiments are needed to determine the grocery store design that maximizes healthful purchasing (in contrast to current stores, which are designed to optimize profits, regardless of the healthfulness of the food purchased).

Second, it will be important to undertake a systematic synthesis of existing evidence to assess the processes that explain the impact of interventions targeting automatic processes. Automaticity remains an elusive concept, difficult to understand and identify (32). The formidable task of conceptually framing and synthesizing study findings is just beginning (33). Such syntheses have the potential to enrich understandings of basic brain processes that activate behavior, as well as to inform the designs of future environments, built and virtual, with a higher chance than existing ones of activating healthier behavior and inhibiting less-healthy behavior on the scale needed to make a measurable impact on population health.

Third, there is scope for the development of new interventions. Individual-level interventions shown to be effective at changing behavior in laboratory and community settings might be adapted so that they can be delivered at the population level. These include computer-based evaluative conditioning procedures that weaken positive associations with potentially health-damaging products (34), training to inhibit behavioral impulses to engage in unhealthy behaviors (35), and the formation of intentions to implement a particular behavioral response upon encountering a particular cue (36). This latter approach would actually capitalize on our remarkable potential to develop automatic responses, in this case putting a positive automatic response in place of a harmful one.

Implementing interventions that target the automatic bases of health-damaging behaviors will require that certain philosophical, political, and economic barriers be overcome. These include the implied threat to our understanding of what it is to be human, given that the most-parsimonious models of behavior involve acknowledging that much of our behavior takes place outside of awareness. Furthermore, given the multitude of existing

cues driving us toward health-harming behavior, it remains to be seen whether we can gain a toehold in an environment that is already exerting strong negative impacts on health. A further threat is posed to economies that are built on excessive consumption, because successful behavioral-based efforts to prevent disease would reduce the consumption of food, alcohol, and tobacco, as well as their transport by fossil fuels. Political and public wills need to be aligned for the successful enactment of interventions that reduce such consumption. Although the precise global strategies for achieving the World Health Assembly’s laudable ambition are evolving, it is clear that behavioral and brain sciences have a role to play.

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## PERSPECTIVE

# Fetal and Early Childhood Undernutrition, Mortality, and Lifelong Health

Chessa K. Lutter<sup>1\*</sup> and Randall Lutter<sup>2</sup>

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Child undernutrition is a broad and complex phenomenon, encompassing fetal undernutrition; insufficient breast-feeding; and complementary feeding of diets low in energy-dense foods, essential fatty acids, and micronutrients. The effects of undernutrition include low birth weight and deficits in height and weight, as well as physiological outcomes later in life. The importance of these factors prompted U.S. Secretary of State Hillary Clinton to describe the benefits of improved nutrition in utero and during the first 24 months of life as providing a valuable “1000 day window of opportunity” for lifelong health and development (6).

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Outcomes	Measure of breast-feeding	Effect size	Notes
Ovarian cancer	Length of breast-feeding	Reduced risk of ovarian cancer by 28% for each year of breast-feeding (odds ratio: 0.72; 95% CI: 0.54 to 0.97)	Meta-analysis of nine studies with 4387 cancer ovarian cancer cases and 10,574 controls (32)
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Hypertension	Never breast-fed versus exclusively breast-fed first child for $\geq 6$ months	Increased risk of hypertension by 29% (hazard ratio: 1.29; 95% CI: 1.20 to 1.40)	55,636 parous women in the U.S., reported 8861 cases during 660,880 person-years of observations (30)



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At the risk of oversimplifying the topic, we review the recent and growing evidence of benefits of early nutrition, particularly breast-feeding, on child mortality and maternal and child health outcomes. We endeavor to distinguish between effects reported in low- and high-income countries, as these effects and the success of interventions may differ with diet and general sanitary conditions. Our review covers research in low- and high-income countries, including observational, case control, prospective cohort, and randomized studies. Our survey indicates there is credible evidence that improved population coverage of child nutrition interventions, particularly related to breast-feeding and complementary feeding, could provide large benefits in absolute terms and that these measures could do so at exceptionally low cost. However, public health funding for child nutrition research and programs is still relatively low compared with that for other life-saving child health interventions (7).

Here, we present evidence for benefits, an economic rationale for government intervention in breast-feeding, and a review of breast-feeding practices and policies. The rest of this paper addresses the early nutritional origins of disease, effective nutrition interventions in the first 1000 days, breast-feeding and NCD risk, the economic rationale for breast-feeding promotion, data on current breast-feeding and complementary feeding practices, and, finally, conclusions.

### Early Nutritional Origins of Disease

The past few decades have seen an explosion of research suggesting that nutrition insults during fetal life have surprising and long-lasting ramifications for health (8, 9). Analysis of such effects is complicated by the lack of accepted measures of in utero exposure, the difficulty separating in utero exposure from exposure during infancy or early childhood, and the possibility of effects sufficiently severe to increase perinatal mortality, thus masking later adverse effects (10). Researchers have addressed these complications by focusing on “natural experiments” such as the Dutch Hunger Winter (resulting from severe wartime food shortages during the winter of 1944–1945) and religious fasts—episodes for which earlier or later cohorts provide suitable controls.

Effects of prenatal exposure to the Dutch Hunger Winter include obesity among 19-year-old men, fat deposition for women, schizophrenia, and elevated blood pressure (10). Prenatal exposure to daytime fasting during Ramadan has been reported to increase the likelihood of adult disability by more than 20% among Iraqis and Uganda’s Muslims, with substantially larger effects for mental and learning disabilities (10). One study considered effects of dietary supplementation with iodine during pregnancy in Tanzania—iodine deficiencies can cause low IQ scores. Before the advent of iodized salt, maternal iodine deficiency was the leading preventable cause of mental retardation globally. After accounting for differences in uptake

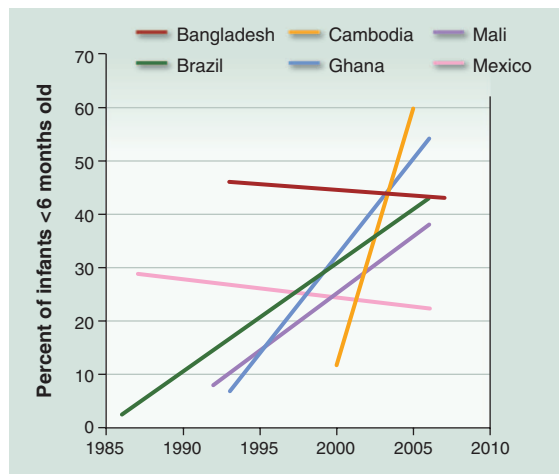
among families, girls who received iodine supplementation in utero were found to have had about an extra 6 months of schooling relative to siblings, even though their health was apparently unaffected (11).

All of these estimates should be seen as illustrative of how nutrition in utero affects long-term health and even schooling, rather than as concrete, quantitative estimates. One reason for this caution is that the biological effects of in utero and early childhood nutritional insults depend on their precise nature, severity, and timing during development. In addition, the effects also probably vary with later diet, physical activity, and genetic predisposition. Another reason for caution is that nonhealth outcomes, such as years of schooling, also depend on how families treat children who may be subtly different, and this probably varies with differences in culture or economic opportunities. Regardless, a growing amount of literature shows that fetal undernutrition, as reflected in size at birth, has been associated with a host of chronic diseases later in life, including coronary heart disease, diabetes, and hypertension (8). Such risks are exacerbated when infants grow up in environments where metabolic disorders are prevalent.

Ongoing prospective cohort studies in Brazil, Guatemala, India, the Philippines, and South Africa show that size at birth and accelerated weight gain after 48 months of life is related to insulin resistance (12), whereas greater weight gain during the first 5 years is associated with elevated blood pressure (13). These damaging effects are more pronounced if children become overweight during later childhood and adolescence. The damaging effects of undernutrition are associated with a wide range of lifetime prospects. For example, among Guatemalan boys living in villages where severe stunting was prevalent, random assignment of infants to high-quality dietary supplementation in the first 2 years of life led to a 46% increase in average wages in adulthood (14). Furthermore, women who were undernourished as children tend to have underweight babies, illustrating intergenerational effects of poor nutrition (15).

### Interventions in the First 1000 Days

A wide variety of policy interventions affecting nutrition in the first 1000 days of life can have long-lasting effects on health. Interventions to prevent child mortality, as highlighted in a *Lancet* series on maternal and child undernutrition in low-income countries, include breast-feeding and complementary-feeding counseling, as well as food supplements (when necessary) in children 6 to 24 months of age (16). Providing vitamin A and zinc supplements, ensuring universal salt iodization, and timely treatment of severe acute malnutrition are all interven-



**Fig. 1.** Selected trends in exclusive breast-feeding, shown as percent of infants less than 6 months of age, inferred from pairs of nationally representative surveys conducted between 1985 and 2010.

tions known to be effective in reducing child mortality (16). Iron supplements are also recommended, but not where malaria is prevalent because of the risk that iron supplements may increase mortality by increasing vulnerability to infections (17).

An important attribute of breast-feeding is that it enhances a child’s IQ, according to numerous studies in high- and low-income countries. A randomized, though unblinded, trial showed that breast-feeding promotion raised IQ six points (18); other studies show gains more on the order of one to three points. Regardless of the exact number, these are large gains, comparable to the well-established effects of eliminating lead from gasoline (19). Analyses by the U.S. Environmental Protection Agency (EPA) suggest that an increase of one point in an individual’s IQ increases the present value of lifetime earnings by between 1.8 and 2.4%. Using data on median earnings of U.S. workers and assuming a discount rate of 3%, the EPA calculates the gain in net earnings from an increase of one IQ point to range from \$8760 to \$12,512 in 2006 U.S. dollars (20). To our knowledge, these estimates have not been included in economic studies of breast-feeding. Because IQ gains are expected in all breast-fed infants, IQ-related benefits appear sufficiently large to substantially improve the cost-effectiveness of breast-feeding interventions relative to other public health measures.

Maternal health matters too, not just intrinsically, but because mothers as the primary care givers for their children need to be physically and mentally healthy to provide adequate care. Indeed, maternal death is a risk factor for infant mortality. Anemia during pregnancy increases a woman’s risk of death from blood loss during delivery (1), and high-quality evidence supports the value of providing iron folate and multiple micronutrient supplements to reduce maternal anemia (16).

In addition, breast-feeding offers substantial benefits to mothers and their families because it

delays the return of ovulation and menses, thereby extending the interval between pregnancies. Less frequent pregnancies reduce neonatal, infant, and child mortality (21) and undernutrition (22). Exclusive, on-demand breast-feeding during the first 6 months after giving birth is as effective at preventing pregnancy as condoms, diaphragms, and oral contraceptives (23). After 6 months, breast-feeding still has substantial contraceptive effects, which are particularly important as the use of active birth-control methods is still low in many countries, particularly in Africa. Nationally representative data on married women of reproductive age in 30 African countries between 2000 and 2012 show that, in more than half of these countries, less than one woman in five used contraceptives. Breast-feeding at current levels, compared with no breast-feeding, is estimated to avert 53 million births per year (24). Using the same data, we estimate that breast-feeding at global levels consistent with the World Health Organization (WHO)'s recommendations would further reduce births by another 12 million annually.

In addition to other benefits, improved basic sanitation (such as hand-washing and access to toilet facilities) can improve child nutrition in low-income countries by reducing intestinal diseases that reduce nutrient absorption and cause loss of appetite (25). A robust body of literature illustrates that the effects of acute illness, particularly diarrhea, in early childhood interact synergistically with poor diet to cause childhood stunting (26). Evidence is

also accumulating to show that nutrition-sensitive agriculture and social protection interventions can positively affect child nutrition (27, 28).

### Breast-Feeding and NCD Risk

Emerging data show that breast-feeding plays a role in reducing NCDs, which in 2010 were estimated to cost \$863 billion globally in medical expenses and lost productivity (29); morbidity and mortality rates from NCDs surpass those from communicable diseases in every region but Africa. Furthermore, women who breast-feed reduce their risk of key NCDs, according to recent observational studies. These women experience lower rates of ovarian and premenopausal breast cancer and type 2 diabetes (Table 1). They also appear to have lower risk of some adverse cardiovascular outcomes (30).

Furthermore, data are beginning to reveal the effects of early childhood feeding patterns on NCD risk to children later in life (31). Systematic reviews of available evidence from low- and high-income countries suggest that children who were breast-fed had lower mean blood pressure and total cholesterol (3), as well as fewer cases of type 2 diabetes (3, 32). However, a more recent study from five prospective cohorts in low- and middle-income countries failed to substantiate these effects (33). Still, the role of different patterns of complementary feeding in relation to risk of NCDs is virtually unexplored (34). Unlike breast milk, which evidence has proven to be superior to other foods for infants, no set of complementary foods or feeding practices is shown to be

of better quality, either for healthy growth in the short term or for lower NCD risk in the long term. However, exposure to high levels of salt early in life may damage developing kidneys, predisposing an individual to subsequent high blood pressure (35). A growing amount of literature suggests that gut microflora may develop differently in response to early introduction of different complementary foods, with potential long-term implications for the host's overall health (36).

### Breast-Feeding Promotion: The Economic Rationale

Among the causes of the global disease burden, communicable diseases have long been the targets of choice. The rationale for intervention is stronger with communicable diseases because of the risks of contagion. Often unrecognized, however, is a comparably strong economic rationale that exists for breast-feeding.

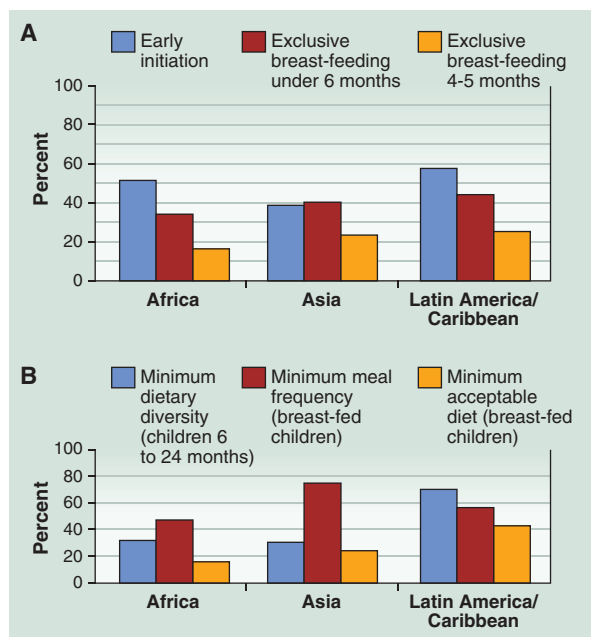
In a 1970 paper that later earned him a Nobel Prize, George Akerlof showed that lower-quality products

can displace higher-quality products in instances where buyers cannot discern quality (37). Such instances of information asymmetry have long been recognized as market failures by the U.S. Office of Management and Budget (38). Breast milk is an example of a higher-quality good whose superiority relative to infant formula is very difficult for mothers to fully perceive. As a nonmarket good, breast milk defies conventional private solutions to information asymmetry problems, such as product warranties or investments in brand-name reputations. Breast-feeding requires successful initiation at birth, when mothers are vulnerable to influence from medical staff or family and ill-placed to make independent decisions. In addition, early use of infant formula hinders later breast-feeding. For mothers who study or work outside the home, breast-feeding requires a place and time for expression and storage of milk, which may be difficult arrangements to negotiate individually with schools or employers. Hence, there is a fundamental and legitimate need for coordinated action to protect breast-feeding, and history reflects recognition of this need.

In the late 1970s, compelling accounts emerged of infants who became acutely malnourished or died from contaminated or diluted formula after free samples were given to their mothers (39). To protect breast-feeding, the World Health Assembly (WHA) adopted the International Code of Marketing of Breast-milk Substitutes in 1981 (40). The code provides guidelines on marketing strategies associated with increased formula feeding, such as direct promotion to the public, free supplies to mothers and health care institutions, and the use of baby images on labels that idealize bottle-feeding. A total of 12 subsequent WHA resolutions—the most recent in 2010—have strengthened the original guidance; nonetheless, violations continue (41). The 1990 Innocenti Declaration endorsed by the WHA set operational targets that governments should achieve, and in 1991, WHO and United Nations Children's Fund (UNICEF) launched the Baby Friendly Hospital Initiative to promote hospital environments conducive to breast-feeding. Elements of successful breast-feeding promotion strategies are well documented (42).

In 1996, breast-feeding promotion was estimated to be exceptionally cost-effective: \$150 for each diarrheal death prevented in Latin America (43). This estimate placed breast-feeding promotion among the most cost-effective interventions for child survival, equal to other high-impact interventions such as immunizations. However, this assessment does not include the gains in IQ and reductions in NCDs, which would make estimates of cost-effectiveness substantially more attractive.

The revolution in information technology could further contribute to efficiencies in breast-feeding promotion. Timely delivery of information that is culturally sensitive, specific to the issue at hand, and authoritative is highly effective in getting mothers to breast-feed exclusively (44). Cell phones or smart phones have been effectively used to communicate



**Fig. 2. (A and B)** Percentage of infants and children meeting recommended breast-feeding and complementary feeding practices. Data are from nationally representative surveys conducted in 46 low- and middle-income countries between 2002 and 2008 and represent 82, 58, and 22% of the population of children younger than 5 years of age in Africa, Asia, and Latin America/the Caribbean, respectively.



health messages in HIV treatment (45) but have rarely been used in programs to promote breast-feeding or complementary feeding. Innovative use of these technologies could greatly improve the cost-effectiveness of child nutrition programs.

Of late, funding for breast-feeding promotion has declined (46). U.S. Agency for International Development (USAID) global spending on child nutrition, of which breast-feeding promotion was an important component, declined from \$16.6 million in 1999 to \$13.3 million in 2003. Between 1999 and 2005, investment in breast-feeding in USAID's flagship maternal and child nutrition project declined from \$4.9 million to \$2.3 million, while project expenditures for prevention of mother-to-child transmission of HIV increased, reflecting the seismic shift in global funding priorities related to the HIV/AIDS epidemic. Donors other than USAID also cut funding (46).

Data from African, Asian, and Latin American/Caribbean countries suggest that supportive policies and programs can markedly affect exclusive breast-feeding percentages (Fig. 1). In 1993, Ghana and nearby Mali had reasonably similar rates (~8%) of exclusive breast-feeding. Yet by 2005, the rates differed by 15 percentage points, despite improvement in both countries. Cambodia achieved a phenomenal gain in exclusive breast-feeding rates of nearly 50 percentage points in 5 years, whereas in Bangladesh, the rate slipped slightly from 46% over 15 years. In Brazil, exclusive breast-feeding increased 40 percentage points, from 3 to 43% between 1986 and 2006, but over roughly the same period in Mexico, the exclusive breast-feeding rate decreased by 5 percentage points. For Brazil, a 20-year chronology links key legislative, policy, and programmatic measures with improved breast-feeding practices (47). Thus, government policy and public health measures appear capable of effecting large gains in breast-feeding in some countries, even given concurrent increases in urbanization, female education, and employment that are traditionally associated with declines in breast-feeding rates.

### Current Breast-Feeding and Complementary Feeding Practices in Selected Low- and Middle-Income Countries

A big gap still separates current practices from accepted breast-feeding recommendations in low- and middle-income countries (Fig. 2) (48). WHO recommends 6 months of exclusive breast-feeding, but current prevalence (36%) is much lower. Only about half of 20- to 23-month-old children are breast-fed, despite the recommendation that all children be breast-fed for 2 years or beyond. Although early initiation prevented about one-fifth of neonatal deaths in Ghana and Nepal (49, 50), less than half of the infants in 46 low- and middle-income countries are put to the breast within 1 hour of birth.

Global practices in complementary feeding in low- and middle-income countries are poor (Fig. 2) (48). Only half of children 6 to 24 months of age met the recommended minimum daily numbers

of meals, less than one-third met the minimum criteria for daily dietary diversity, and only one in five breast-fed children satisfied the criteria for minimum acceptable daily diet. Moreover, there are wide differences among countries with relatively similar income levels. In Ethiopia, which had a gross domestic product (GDP) of \$1100 in 2011, only 3.9% of children 6 to 24 months of age met the minimum standard of daily dietary diversity. In contrast, in Uganda, with an estimated GDP of \$1300 for same year, 23.6% of children in the same age group satisfied this criteria (51). Low national income, though important, is not the only impediment to improved complementary feeding.

### Conclusions

The prenatal period and the first 24 months of life provide a 1000-day window in which sound nutrition, especially adherence to recommended breast-feeding and complementary feeding practices, can improve not only the health of vulnerable infants and young children, but also the trajectory of aspects of their well-being and the health of their mothers. However, a large gap between current and best practices exists. Research on how to cost-effectively improve the coverage of existing nutrition interventions is needed to help accelerate their health impacts (7).

Research is also needed to better understand the biological mechanisms through which the effects of improved breast-feeding occur, because randomization in breast-feeding studies is nearly impossible to achieve. Most evidence derives from observational studies whose interpretations are complicated by self-selection, measurement errors, and residual confounding (3). Knowledge of the underlying metabolic pathways through which breast-feeding or breast milk affects specific health outcomes, such as the role of human milk serum adiponectin exposure and early childhood weight gain (52) and how human milk and complementary foods affect the gut microbiome, will improve interpretation of epidemiological studies.

Acquiring a deeper understanding of the most common breast-feeding and complementary feeding difficulties and identifying the most effective strategies to overcome these difficulties is essential. Surveys, randomized interventions, and systems analyses are needed to explore the functioning of health care systems and the behavior of health professionals in relation to the persistence of impediments to better feeding practices. Both basic and applied research are required to develop an evidence-based set of policies and programs to improve complementary feeding. Finally, research is needed to measure the population risk attributable to sub-optimal feeding practices and child nutrition, as well as the costs in medical treatment and lost productivity.

The beneficial effects on child mortality and IQ and on maternal NCD risks of improved nutrition during the prenatal period and first 2 years of life appear large compared with other public health interventions. Because breast-feeding promotion provides the greatest short-term benefit for children living in poor environments, investments in breast-

feeding protection and promotion will also improve global health equity. Nonetheless, funding for research and greater use of existing effective interventions is low compared with other life-saving child health interventions.

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## PERSPECTIVE

# Double Burden of Noncommunicable and Infectious Diseases in Developing Countries

I. C. Bygbjerg

On top of the unfinished agenda of infectious diseases in low- and middle-income countries, development, industrialization, urbanization, investment, and aging are drivers of an epidemic of noncommunicable diseases (NCDs). Malnutrition and infection in early life increase the risk of chronic NCDs in later life, and in adult life, combinations of major NCDs and infections, such as diabetes and tuberculosis, can interact adversely. Because intervention against either health problem will affect the other, intervening jointly against noncommunicable and infectious diseases, rather than competing for limited funds, is an important policy consideration requiring new thinking and approaches.

In 1971, Omran (1) described how health and disease patterns change over time in societies, depending, among other factors, on the degree of demographic transition and rate of economic development, to result in an epidemiological transition. Like individuals, societies have a “life cycle”: In a “young” society, infectious diseases and nutritional deficiencies dominate; hence in children, diarrhea and acute respiratory infections, including measles and malaria, predominate; in pregnant women, fetal loss, perinatal death from undernutrition, bleeding, and infection are major risks, and in the surviving adults, tuberculosis (TB) and other diseases related to poverty are important causes of morbidity. When societies “grow up,” accidents and violence-related disabilities and deaths increase, mostly among the young, and although infectious diseases such as TB still prevail, chronic noncommunicable diseases (NCDs) become more prevalent, particularly in urban populations. In aging societies, NCDs predominate: first, type 2 diabetes and cardiovascular diseases, followed by cancer and degenerative disorders. Simultaneously, in extreme cases the broad-based demographic pyramid inverts.

Demographic transition as the main explanation for the growing NCD burden has, however, been questioned. Stuckler (2), in a thorough analysis of

causes that was published in the same journal as Omran's historical paper, pointed out that particularly in low-income countries, economic growth, market integration, foreign direct investment, and urbanization together correlated threefold greater to epidemiological transition than did population aging. The projections of disease burden in Fig. 1 are made by considering the combined effect of demographical transition (population growth and increasing life expectancies) and expected impact of changing lifestyle, living conditions, and economic development (3).

Omran (1) has also been criticized for overlooking new epidemics of infectious diseases, but this author could not have predicted the HIV epidemic, which disturbed his model, set back the epidemiological and demographic transition, and, more importantly, reversed the reduction of deaths from infections in children and young adults, particularly in sub-Saharan Africa. As a consequence, combating HIV and other major infections and improving child and maternal health remained prominent among the Millennium Development Goals (MDGs) set in 2000. Similarly, the United Nations' General Assembly (UNGASS) in 2001 committed all governments to combat the HIV epidemic (but not NCDs), and, consequently, WHO and UNAIDS updated their “Strategies for the Prevention and Control of Communicable Diseases” (4). In that document, NCDs—such as diabetes, as a potential risk factor for infections, or TB in particular—were not mentioned. None of the MDGs relate directly to NCDs,

although Stuckler *et al.* (5) and others have indicated that MDGs may not be attained without addressing NCDs as risk or cofactors for communicable diseases.

Before the turn of the millennium, some researchers (6) and the World Health Organization (WHO) (7) had pointed at the danger of a “double burden of disease,” such as the emerging epidemic of chronic NCDs, in addition to the “unfinished agenda of infectious diseases” and problems of maternal and child health. Yach *et al.* (8) showed that even in the poorest countries, more deaths are caused by NCDs than from infections, and that the WHO Headquarters spent only US\$0.50 on chronic diseases per death per person compared with US\$7.50 for leading communicable diseases. Yet in 2005, the WHO in its report “Preventing chronic diseases—A vital investment” (9) underscored that NCDs do not only hit the old, the rich, and the fat; developing countries carry the heaviest burden of NCDs. In 2007, the World Bank (WB) issued a similar report on the conceptions, misconceptions, and challenges presented by chronic NCDs (10). In 2011, partly as a result of these reports and provoked by continuous lobbying by civil society and leading stakeholders in NCDs, including the International Union Against Cancer, the World Heart Federation, the Global Alliance against Chronic Respiratory Diseases, the International Diabetes Federation, and the International Union Against Tuberculosis and Lung Disease, UNGASS committed governments to fight the emerging epidemic of NCDs, acknowledging that NCDs hit developing countries hard (11). When reading and comparing the UNGASS declarations from 2001 and 2011, unfortunately, the known and potential links between infectious diseases and NCDs are barely visible. Similarly, in the 182-page 2005 WHO report (9) and the 188-page 2007 WB report (10) on NCDs, TB is mentioned once in each report, malaria once in the WHO report, and HIV six and three times, respectively.

A major barrier for integrated intervention against the double burden of infections and NCDs may be that their etiologies and pathologies at first glance appear to be diametrically opposed. As part of new public management, researchers, health professionals, donor agencies, and politicians are often forced to focus on a particular health problem to get visible results and fulfill result contracts. At a time of global financial crises and shrinking health budgets, there is a threat that the battle against common

Copenhagen School of Global Health, Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, 5 Øster Farimagsgade, DK-1014, Copenhagen K, Denmark. E-mail: iby@sund.ku.dk.

and future health problems, both communicable and noncommunicable, may become a fight for funds to control either health problem, rather than a fight against the double burden of disease.

A key question to be answered before setting priorities and allocating (or reallocating) resources for health beyond 2015 is whether the sharp demarcation between communicable diseases and NCDs, which is apparent in most projections of global mortality and burden of disease—including WHO's in Fig. 1—is justified when both may hit the same individuals and societies. In the following, I highlight some potential and verified links between diseases and discuss possible ways of addressing them jointly and/or without unnecessary competition for financial or human resources.

Intensifying interventions against joint risk factors for NCDs—including diabetes, chronic obstructive pulmonary disease, some cancers, and against a major infectious disease, TB—by fighting malnutrition (12) and tobacco and alcohol use is an obvious priority. Preventing common cancers by vaccinating against the virus that induces them, including human papilloma virus (HPV) and hepatitis B (HBV), is another priority.

If NCDs could be prevented by improving maternal and child health, it would be welcomed by policy- and decision-makers in low- and middle-income countries (LMICs). But how much are NCDs in adults related to ill-health in mothers and children? Indeed, infection and deficiencies in pregnancy, such as malaria and anemia, often result in fetal and maternal loss, prematurity, and low birth weight (13), and it is established that undernutrition and communicable diseases in childhood do interact (14). It is less well known that adverse events in early life play a major role in cardiovascular diseases and diabetes. In the 1980s, Barker and Osmond (15) found that ischemic heart disease mortality rates strongly correlated with neonatal and post-neonatal mortality 50 years earlier. This prompted Hales and Barker's "thrifty phenotype theory," now confirmed, that low birth weight may induce early debut of cardiovascular disease and type 2 diabetes (16), as also described by Lutter and Lutter in this issue (17). Thus, intensifying programs to improve maternal and child health could reduce risks for NCDs. As we have proposed, and presently are investigating in East Africa, metabolic disease in adult life may even be prevented by malaria control in pregnancy (18). Barker and Osmond (15) showed that poor nutrition in early life increases susceptibility to the effects of a more affluent diet in later life. In many LMICs, a nutritional transition may take place in a single generation, and it is possible that induction of insulin resistance by epigenetic silencing of insulin-regulating genes in utero could be the mechanism (19). Insulin resistance induced in early life may be reversible, but if catch-up growth is too fast, some data point at an increased risk of low-birth-weight children becoming obese (20). Breast-feeding is strongly recommended for low-, as well as normal-, birth-weight and large

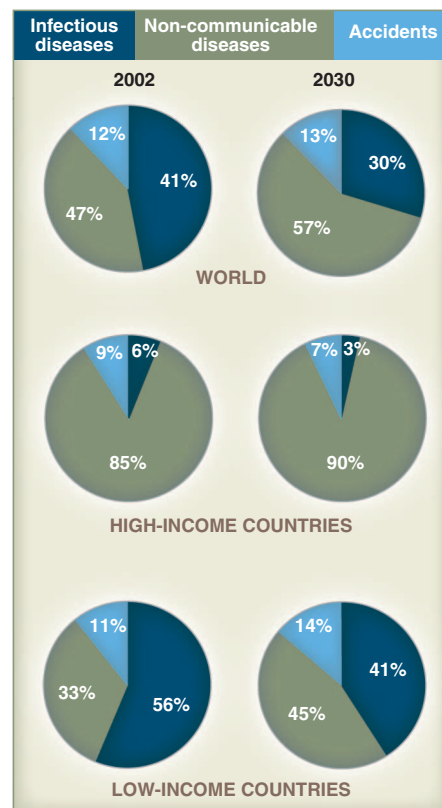
(macrosomic) babies and protects against diarrhea, respiratory infections, and obesity (21). Overweight mothers and high-birth-weight neonates are at high risk for developing diabetes (22). Promotion of breast feeding would be a low-cost, low-hanging tool for the prevention of NCDs, as well as infection (17).

Screening for gestational diabetes could be part of an integrated, antenatal care program, along with screening for and prevention of malaria, HIV, and HBV transmission from mother to child. HIV increases the risk for HPV-induced cervical cancer threefold, and 80% of cervical cancer cases occur in LMICs (23). Presently, highly effective but costly vaccination against HPV is mainly available in high-income countries, although vaccination against HBV is increasingly becoming part of expanded programs on immunization, even in LMICs, preventing cirrhosis, as well as cancer of the liver.

The HIV epidemic taught us that the risk for developing TB increases more than fourfold where both diseases are prevalent. In spite of this, it took almost 20 years before the WHO and UNAIDS managed to agree on guidelines for the implementation of collaborative TB and HIV program activities (24). HIV plus malaria also negatively affect human health; likewise, it took 20 years to formulate recommendations on how to fight them jointly (25). Hopefully, strategies for combating the double burden of communicable diseases and NCDs have a shorter incubation time. There are some success stories.

Diabetes' interaction with TB was recognized early but later forgotten by clinicians and public health experts when TB declined in the Western world. Only when diabetes rose exponentially in TB-prevalent LMICs were interactions and implications for control reviewed (26). This review and other publications documented that the risk for developing TB increases threefold in diabetics, and that TB may in turn increase the risk for type 2 diabetes by inducing glucose intolerance and deteriorating glycemic control (27). Fortunately, the lessons learned from the HIV-associated TB prompted a much faster reaction to the looming epidemic of diabetes-associated TB (28). A framework has been established in less than 5 years to guide national programs, clinicians, and others engaged in care of patients on how to establish a coordinated response to both diseases at organizational and clinical levels. In China, India, and other countries with high burdens of both diseases, large-scale programs for dual screening and management are now being rolled out (29). One example of applying management tools from TB directly to the management of diabetes in a low-income country is the successful introduction of "DOTS for Diabetes" in Malawi (30).

As Halldan Mahler, "the father of primary health care" stressed back in 1966 (31), when attempting to convince decision-makers that a double burden of disease requires integrated control strategies that should begin in the primary health care sector (32), it should not be forgotten that "integration, far from being a laissez-faire approach, requires maximum involvement of all specialized personnel such as



**Fig. 1.** The proportional distribution of disability-adjusted life years, contributable to infectious diseases and NCDs for (top) the world, (middle) high-income countries, and (bottom) low-income countries for 2002 and 2030 (3).

programmers, organizers, tutors and assessors." Thus, when adjusting health systems to meet the challenges of the double burden of disease, whether caused by aging or changing living conditions or both, a multidimensional approach is needed, ranging from education, equity, and economic development to environmental change (33).

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## PERSPECTIVE

# Why a Macroeconomic Perspective Is Critical to the Prevention of Noncommunicable Disease

Richard Smith

Effective prevention of noncommunicable diseases will require changes in how we live, and thereby effect important economic changes across populations, sectors, and countries. What we do not know is which populations, sectors, or countries will be positively or negatively affected by such changes, nor by how much. Without this information we cannot know which policies will produce effects that are beneficial both for economies and for health.

Bill Shankly (manager of Liverpool Football Club from 1959 to 1974) said football (soccer) is “not just a matter of life and death, it’s more important than that.” For economists, so are noncommunicable diseases (NCDs) (1). Not only are the effects of NCDs felt throughout the economy (Table 1), but since the agents contributing to NCDs are influenced by our lifestyles, effective preventive policies are likely to include mechanisms that themselves have appreciable economic impacts, such as taxing soft-drinks, increasing the use of public transport, or promoting lower-polluting energy sources (2, 3). Although the impacts of such policies may improve health, there will be substantive economic impacts as they ripple out through the economy, generating differential effects across various sectors, such as housing, transport, and agriculture. These economic effects may generate yet further health effects, which themselves then feed into the economy, generating yet more cycles of effects. This interaction and reciprocity between NCDs and the economy highlights the critical need for a macroeconomic perspective in the design, implementation, and evaluation of preventive policies to tackle NCDs.

Macroeconomics, as compared with microeconomics (which is focused upon “partial equilibrium” within a single sector, such as for housing or meat), is concerned with general equilibrium across all sectors, and thus how changes in one sector (e.g., increase in price) affect other sectors, with all these changes together comprising the overall “economic impact” of a single change (4, 5). For instance, the impact of pandemic influenza on the healthcare sector is minimal compared with its effect on gross domestic product (GDP) through impacts on other sectors (e.g., hotels, leisure, travel), which are a consequence of changes in individual behavior in response to pandemic threat and the mitigation policies themselves (6, 7).

## Why Is This Important for NCD Prevention?

NCDs, such as diabetes, cancer, and heart disease, differ from infectious diseases, such as pandemic influenza, as they are not transmitted from person to person [although there is evidence emerging in the social sciences of “social contagion,” where social networks appear to influence the probability of obesity, for instance (8)]. However, they also differ in that they are intrinsically lifestyle diseases, and hence the cause and impact are linked in a multiplicity of ways to everyday economic activity (Fig. 1).

NCD-related health (Fig. 1, box 1) is determined directly by risk factors (Fig. 1, box 2), which include genetic predisposition to disease,

such as diabetes and heart disease, but also by a range of other social determinants of health, which refer to the general conditions in which people live and work, including levels and types of employment, environmental conditions, and education (9). These social determinants, contribute to the risk of different diseases, such as pollution-related diseases and cancer. They are also intimately linked with the household and individual (Fig. 1, box 3), which represent how people behave and, crucially, invest (or disinvest) in their health by what they consume and in the activities they undertake (8). For example, cancer and heart disease risk will be affected by decisions concerning smoking, alcohol consumption, and exercise. But risk will also influence household and individual behavior. For instance, an individual’s knowing that they have a higher genetic risk of heart disease may modify individual consumption of fast food. The healthcare sector (Fig. 1, box 4) comprises goods and services consumed by households principally to improve health status. Although these affect NCD-related health directly, they also impact on the household economy, which ultimately pays for them through taxation, insurance, or out-of-pocket. The level of ill-health caused by NCDs will also feed back and impact on the household, thus further affecting the risk of other health problems through reducing household income, and feed into healthcare provision through shaping the demand for services, and hence profile of provision (e.g., more insulin prescriptions).

Activity in all non-healthcare sectors in the economy (Fig. 1, box 5), such as agriculture, manufacturing, and education, impacts on the previous three components and, thus, NCDs. It is well established, for instance, that “wealthier is healthier” (10, 11), but that wealth also brings an increase in NCD risk, such as through changes in dietary habits, with the suggestion that in some cases this means that economic recessions can have positive health benefits (12). As countries grow wealthier, their populations experience increased desirability and availability of processed foods, perhaps mostly starkly indicated by the experience of some Pacific island populations where traditional diets have been displaced with high-fat imported foodstuffs and a concomitant increase in obesity rates and NCDs. Similarly, the transformation of food retail as countries become

Health System Economics, Faculty of Public Health and Policy, London School of Hygiene and Tropical Medicine, 15-17 Tavistock Place, London WC1H 9SH, UK. E-mail: [richard.smith@lshtm.ac.uk](mailto:richard.smith@lshtm.ac.uk)

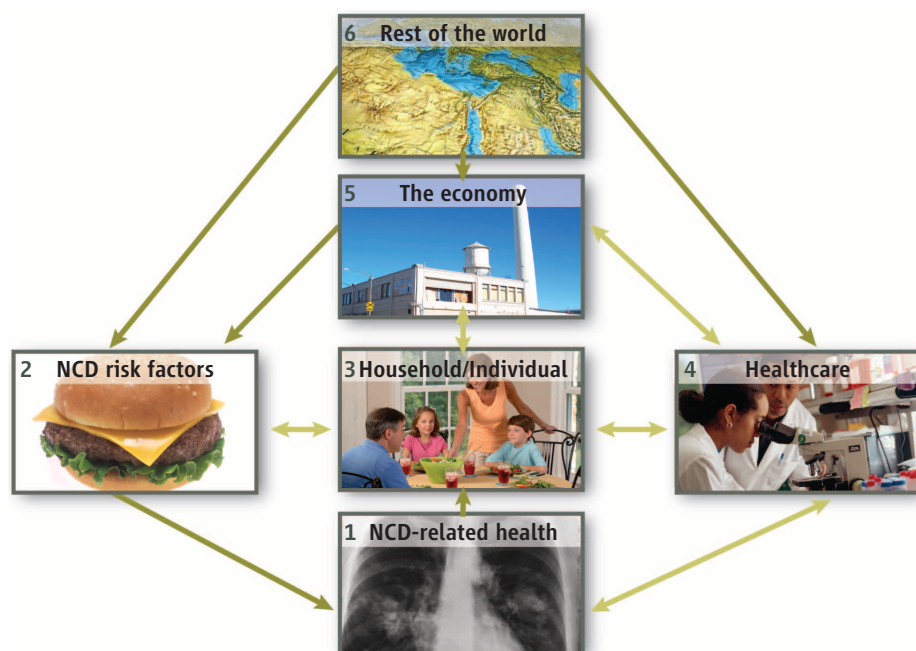


Fig. 1. Noncommunicable diseases and the macroeconomy.

more integrated in the global trading system has facilitated a pronounced shift to the consumption of processed food, and multinational fast-food outlets have made substantial investments in growing economies (13). It is also accepted that health positively affects the general economy through a fitter, more educated, and more productive workforce (14). Insecurity, as labor moves from one sector or location to another, generates ill-health directly through the stress caused by economic and social dislocation, and indirectly by increasing poverty (15). Economic well-being, tax rates, and other aspects also affect healthcare spending.

Finally, influences beyond our political borders act on these components (Fig. 1, box 6). For instance, climate change is a global issue with local consequences for NCD risk factors. Problems with subprime lending in the United States, or the Euro crisis, will affect household employment, income, and inflation in the United Kingdom, too. The migration of health professionals affects the ability to provide services required to treat NCDs.

### Prices Are Pivotal

For economics, prices are pivotal. They enable exchange of goods and services and, crucially, determine the point of equilibrium, where demand and supply are balanced. Any change within the economy will affect price directly or indirectly, which will then disrupt this balance, and each sector will then have to adjust to a new equilibrium through changes in other prices. It is a simple, largely automatic, system that has profound implications for NCD prevention—illustrated by the current enthusiasm for a “fat tax” as a mechanism for reducing consumption of foods high in saturated fat through

increasing their price. Such a tax has been implemented in Denmark and Hungary and is now being considered by many others (16, 17).

The argument is simple. The current price of foods high in saturated fat does not adequately reflect the negative health effects, leading to overconsumption (compared to a hypothesized equilibrium where such health effects were captured in the price). Government can apply a tax to address this “market failure,” which increases price. We know that, in general, increasing price leads to a reduction in demand, which reduces consumption, and thus should reduce rates of NCD. Microeconomic, partial-equilibrium analysis will tell us how sensitive this demand is to a change in price (which we know is actually not very sensitive in the case of most foods) and thus by how much price needs to increase (usually, therefore, a lot) (16). The problem, from a macroeconomic perspective, is that it does not tell us anything at all about how other sectors will adjust and thus we cannot know what the overall net impact on the wider economy, or even health, will actually be.

Consider an example where such a tax increases the price of beef. How might consumers react? Any change in price will cause a recalibration of expenditure across the range of goods and services that individuals consume, not just beef. At the extremes, consumers may either reduce spending on beef, to keep spending on everything else the same, or spend less on something else to keep beef consumption the same. What are the implications of these two scenarios?

If consumers reduce their demand for beef, then beef farmers will experience reduced in-

come. Remember that the price is higher as a result of a tax, and hence this extra revenue per unit of beef goes not to the farmer, but to the government—generating further questions about what this tax revenue would be spent on, such as subsidizing fruit and vegetables versus reducing income tax, and the implications of that, which we don’t have time to consider here. As beef is less attractive to produce, farmers will transfer production to other products. Which other products is then the critical question. If it is biofuels, for example, this may have positive effects on the environment and thus further decrease risk factors for NCDs and multiply the health effect. Alternatively, if farmers switch to producing lamb, the increased supply will mean that the price of lamb will fall, and it may largely replace beef in the national diet, negating health benefits from reduced beef consumption.

Farmers could instead focus on increasing exports of beef, thus increasing consumption, and rates of NCDs, in other countries and effectively “exporting” the health problem. What if the beef consumed in one country is imported from another? In this case, it is possible that a tax may not be able to be levied, as it may violate World Trade Organization requirements if the country is a member state. But if a “fat tax” could be levied, then there are economic advantages as less income would be transferred overseas as beef imports declined (which will affect balance of payments and currency valuation with further spill-over effects). However, again there could be negative health and economic implications for the countries from which the beef was imported. For example, although changing to a healthy diet may be beneficial for the United Kingdom, if this is achieved through reduced imports of beef, then Brazil (as the world’s largest exporter of beef) may see a substantive negative impact on its economy, and consequently its population’s health (18).

But what if consumers keep consuming beef, and instead spend less on something else? If less is spent on fruit and vegetables, for example, then this could make health worse. Alternatively, consumers may spend less on car travel, which could have further positive health benefits from reduced emissions, or spend less on leisure activities, possibly having negative health implications from reduced exercise, and certainly having economic implications for those sectors. Or they may spend less on flat-screen televisions or computer games, perhaps generating positive health effects if this leads to increased active leisure pursuits. As above, this spending reduction may affect imported goods, generating effects on the balance of payments of other countries and exchange rates. The ripples continue.

Thus, we know that such a food tax would impact directly on consumption patterns, but after that we know little about what will happen. A food tax will affect the risk of NCDs in an unpredictable manner as it begins to indirectly influ-

ence other sectors in the national economy and interface with the rest of the world. If the net effect is to increase health, then this should feed positively into the economy itself, by reducing healthcare costs and by improving workforce productivity. However, we do not know that this will be the effect, because we do not consider the broader macroeconomic picture.

### What Is the Solution?

We know that a more comprehensive and integrated economic approach is required for developing optimal strategies for preventing and coping with NCDs; the health sector alone cannot achieve the required reduction in NCDs. We also know that there will be differential effects from these strategies across populations, sectors, and countries. What we do not know is which populations, sectors, or countries will be positively or negatively affected, or by how much.

As indicated above, to generate the most effective and acceptable policies to improve NCD prevention there is need to engage with macroeconomic factors to generate optimal prices, subsidies, safety nets, trade agreements, and so forth if a country decides it is advantageous to nudge its population toward healthier behavior. This presents several challenges, such as the specification of causal pathways and mechanisms to reconcile

and balance non-health (e.g., employment) versus health outcomes. Thus, to make the unknowns known will require a substantial paradigm shift in academic, professional, and policy circles (19). Critically, studies concerning the whole-economy effects at a global level are required. Current evidence tends to focus either upon the broad, general, effect of changes in disease upon the economy, or of changes in the economy upon disease (12), or focus upon a specific sector (e.g., studies concerning “fat taxes” tend to consider the impact of a price increase only on the food of interest) (16). Very few studies consider the cross-sectoral or cross-country causes or impacts of NCDs, and measures that may be used to prevent NCDs, or integrate the economic and health effects (18). Yet without this information, we cannot know which policies will produce net beneficial effects, for the economy or health, or what countervailing policies may be required to minimize negative spillovers.

Because NCDs affect the economy so profoundly and pervasively, we also need to quantify these effects, as it is often the economic case that swings the agenda and mobilizes resources. The history of communicable disease, in this respect, provides valuable lessons. The economic impact of HIV/AIDS, tuberculosis, and malaria in particular was important in mobilizing initiatives

such as the President’s Emergency Plan for AIDS Relief and The Global Fund to Fight AIDS, Tuberculosis and Malaria. This was due in part to the WHO Commission on Macroeconomics and Health in 2000, which established firmly that investments to reduce such diseases would be a primary driver of macroeconomic development (14). Having HIV/AIDS as the first health-focused UN high-level meeting in 2001 was also prompted by the devastating effect the virus was having on African economies (14). The second health-focused UN high-level meeting on the NCD challenge, in 2011, also stressed the economic impact of chronic disease (1). With the resolution of the 65th World Health Assembly in 2012 to reduce premature deaths from NCDs by 25% by 2025, the imperative now is to formulate strategies to achieve this target, which requires us to recognize that NCD prevention is “not just a matter of life and death, it’s more important than that.”

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**Table 1.** Projected foregone national income due to heart disease, stroke, and diabetes, 2006 to 2015 (20).

	Foregone GDP (U.S. \$ billions)		Cumulative GDP loss (U.S. \$ billions) by 2015
	2006	2015	2015 (as a percentage of 2006 estimates)
China	1.01	1.84	182
India	1.35	1.96	145
Russia	1.49	1.64	110
Brazil	0.33	0.50	150
Indonesia	0.33	0.53	158
Mexico	0.48	0.89	186
Turkey	0.39	0.52	133
Pakistan	0.15	0.21	140
Thailand	0.12	0.18	150
Bangladesh	0.08	0.14	175
Ukraine	0.13	0.13	100
Egypt	0.11	0.14	125
Argentina	0.13	0.16	125
Burma	0.03	0.06	200
Iran	0.08	0.13	167
Poland	0.17	0.23	133
South Africa	0.16	0.21	133
Philippines	0.06	0.07	133
Colombia	0.07	0.10	150
Vietnam	0.02	0.03	200
Nigeria	0.12	0.12	100
Ethiopia	0.03	0.03	100
DR Congo	0.00	0.03	140
<b>Total</b>	<b>6.8</b>	<b>9.8</b>	<b>83.8</b>



# Neighborhood Effects on the Long-Term Well-Being of Low-Income Adults

Jens Ludwig,<sup>1,2\*</sup> Greg J. Duncan,<sup>3</sup> Lisa A. Gennetian,<sup>4</sup> Lawrence F. Katz,<sup>2,5</sup> Ronald C. Kessler,<sup>6</sup> Jeffrey R. Kling,<sup>2,7</sup> Lisa Sanbonmatsu<sup>2</sup>

Nearly 9 million Americans live in extreme-poverty neighborhoods, places that also tend to be racially segregated and dangerous. Yet, the effects on the well-being of residents of moving out of such communities into less distressed areas remain uncertain. Using data from Moving to Opportunity, a unique randomized housing mobility experiment, we found that moving from a high-poverty to lower-poverty neighborhood leads to long-term (10- to 15-year) improvements in adult physical and mental health and subjective well-being, despite not affecting economic self-sufficiency. A 1-standard deviation decline in neighborhood poverty (13 percentage points) increases subjective well-being by an amount equal to the gap in subjective well-being between people whose annual incomes differ by \$13,000—a large amount given that the average control group income is \$20,000. Subjective well-being is more strongly affected by changes in neighborhood economic disadvantage than racial segregation, which is important because racial segregation has been declining since 1970, but income segregation has been increasing.

Nearly 9 million people in the United States live in “extreme-poverty” neighborhoods in which at least 40% of residents have incomes below the federal poverty threshold, which for 2011 equaled about \$23,000 for a family of four (1, 2). Such neighborhoods also tend to be racially segregated, with high rates of crime and disorder and low-quality public services (3). Studies dating back as far as the 17th century have shown that people living in distressed neighborhoods have greater criminal involvement and fare worse on educational, economic, and health outcomes than do those living in less distressed areas (3–6). These patterns have generated a long-standing concern that distressed neighborhood environments might themselves adversely affect people’s lives and “doubly disadvantage” their low-income residents.

But much uncertainty remains about the degree to which variation across neighborhoods in people’s outcomes reflects the independent causal effects of neighborhood environments per se instead of the propensity of different types of people to live in different areas. Even the most detailed data collection effort may be unable to

measure adequately all of the individual- or family-level characteristics that influence both neighborhood selection and life outcomes. This type of “selection bias” can substantially distort nonexperimental estimates of “neighborhood effects” (7). Yet, determining the importance of changes in people’s neighborhood environments for their life outcomes is a central issue for the social and medical sciences and social policy.

An understanding of the mechanisms through which neighborhood environments affect people’s lives is a crucial issue for policy design. Much of the debate among researchers has focused on the relative importance of residential racial segregation versus economic segregation. Nearly 70 years ago, Gunnar Myrdal argued that racial segregation enabled policymakers to reduce the quality of public services to blacks without harming whites (8), a concern echoed by the 1968 National Advisory Commission on Civil Disorders (the “Kerner Report”) (9). Douglas Massey and Nancy Denton subsequently argued in their widely cited 1993 book *American Apartheid* that “residential segregation has been instrumental in creating a structural niche within which a deleterious set of attitudes and behaviors—a culture of segregation—has arisen and flourished” ([10], p. 8).

In contrast, William Julius Wilson’s landmark 1987 book *The Truly Disadvantaged* argued that the flight of black working- and middle-class families out of ghettos in the 1960s and 1970s was harmful to the families who remained behind not because of any increased racial segregation, but rather because this exodus removed “mainstream role models that help keep alive the perception that education is meaningful, that steady employment is a viable alternative to welfare, and that

family stability is the norm, not the exception” ([11], p. 49). Subsequent work has examined other pathways through which spatially concentrated disadvantage might affect people’s lives, such as declines in “collective efficacy”—the willingness and ability of community residents to work together to support shared norms (3, 5).

Distinguishing the effects of changes in racial versus income segregation also helps answer the question of whether the problem of harmful neighborhood effects on disadvantaged populations is getting better or worse over time, given opposing recent trends in U.S. residential segregation by race and income. Specifically, racial segregation in America peaked in 1970 and has been declining over the past 40 years, to levels not seen since 1910 (12), whereas income segregation has been increasing since 1970 (13, 14).

This paper examines the long-term effects of moving into a less distressed neighborhood environment on the well-being of low-income adults using new data from a unique, large-scale randomized social experiment: the U.S. Department of Housing and Urban Development’s (HUD) Moving to Opportunity (MTO) demonstration. Via random lottery, MTO offered some public housing families but not others the chance to move into a less distressed area (supplementary materials, section 1). MTO randomization generates large, persistent differences in neighborhood conditions across otherwise comparable groups of families and enables us to attribute differences in post-baseline outcomes across groups to the MTO-assisted moves.

Unlike many social experiments that follow people for short periods, we focused on long-term effects through in-person data collected 10 to 15 years after randomization. We have shown elsewhere that MTO moves have long-term beneficial effects on a narrow but important set of physical health measures, related to extreme obesity and diabetes (15). The implications for how neighborhoods affect the overall quality of the lives of participating families were not addressed in that work.

We used data from the MTO experiment to examine the long-term effects of moving to less distressed neighborhoods on broad measures of the well-being of low-income adults. We examined “objective” outcomes (economic self-sufficiency, physical health, and mental health) that have been the traditional focus of this literature. We also took a new approach in examining experimental neighborhood effects on a comprehensive measure of people’s quality of life as they perceive it, using adult self-reports of subjective well-being (SWB). And, we investigated the relative importance of racial segregation versus income segregation in affecting the SWB of low-income adults.

**The MTO experiment.** From 1994 to 1998, MTO enrolled 4604 low-income public housing families living in high-poverty neighborhoods within five U.S. cities: Baltimore, Boston, Chicago,

<sup>1</sup>Harris School of Public Policy, University of Chicago, 1155 East 60th Street, Chicago, IL 60637, USA. <sup>2</sup>National Bureau of Economic Research, 1050 Massachusetts Avenue, Cambridge, MA, 02138, USA. <sup>3</sup>School of Education, University of California, Irvine, 2056 Education Building, Mail code 5500, Irvine, CA 92697, USA. <sup>4</sup>The Brookings Institution, 1775 Massachusetts Avenue NW, Washington, DC 20036, USA. <sup>5</sup>Department of Economics, Harvard University, Cambridge, MA 02138, USA. <sup>6</sup>Department of Health Care Policy, Harvard Medical School, 180 Longwood Avenue, Boston, MA 02115, USA. <sup>7</sup>Congressional Budget Office, 2nd and D Streets SW, Washington, DC 20515, USA.

\*To whom correspondence should be addressed. E-mail: jludwig@uchicago.edu

Los Angeles, and New York. Families were randomized into three groups: (i) the Low-Poverty Voucher (LPV) group, which received housing vouchers that subsidize private-market rents but could only be used in census tracts with 1990 poverty rates below 10%; (ii) the Traditional Voucher (TRV) group, which received regular housing vouchers without any MTO relocation constraint; and (iii) a control group, which received no assistance through MTO. Some 48% of the adults assigned to the LPV group and 63% of those assigned to the TRV group managed to relocate using an MTO voucher (the MTO “compliance rate”). Because the effects of LPV and TRV assignment on neighborhood conditions converge over time, and to maximize statistical power, we initially present results that pool the two treatment groups together. (Separate estimates for the LPV and TRV groups are in tables S1 to S4.)

Data from baseline surveys collected from all MTO adults shows these families were quite economically disadvantaged when they applied for MTO (Table 1). Most household heads were African-American or Hispanic females; less than 40% had completed high school. By far the most common reason applicants reported signing up for MTO was to get away from gangs and drugs, with around three quarters reporting this as one of their top two reasons for wanting to move.

Being a random assignment experiment, the distribution of baseline characteristics was balanced between the treatment and control groups. Among the 21 baseline characteristics reported in Table 1, only two treatment-control differences are significant at  $P < 0.10$ , and none is significant at the  $P < 0.05$  threshold. An  $F$  test fails to reject the null hypothesis that treatment-control differences in the baseline variables shown in Table 1 are jointly zero ( $P = 0.462$ ).

**Measures.** To measure long-term effects of changing neighborhoods on adults in the MTO demonstration, the Institute for Social Research at the University of Michigan—under subcontract with our research team—collected in-person data from participants 10 to 15 years after random assignment (hereafter “long-term survey”) (supplementary materials, section 2). Interviewers were blinded to the MTO group assignments of participating families. The effective response rate for MTO adults was 90% and was similar across randomized MTO groups.

To measure the neighborhood conditions in which families were living during the follow-up study period, we linked address information for MTO adults to census tract-level data on population characteristics from the 1990 and 2000 decennial censuses and the 2005 to 2009 American Community Surveys. Our main results focus on duration-weighted tract characteristics averaged over the entire post-randomization study period because people’s life outcomes may depend on cumulative exposure to neighborhood environments, not just current neighborhood conditions (16). The long-term surveys also asked MTO participants to self-report on conditions of the neigh-

borhoods and housing units in which they were living at the time.

To measure neighborhood effects on traditional “objective” measures of well-being, we constructed summary indices of long-term adult outcomes in the domains of economic self-sufficiency, physical health, and mental health. We focused on adults in part because of our interest in well-being over the long term, which may not yet be evident for the MTO children. Our outcome indices are constructed from a set of individual outcomes that are rescaled so that higher values represent “better” outcomes and then converted to  $z$  scores by using the control group distribution. Aggregating outcomes improves statistical power to detect impacts and reduces risk of “false positives”

from examining numerous outcomes (7). To reduce the risk of false positives due to data mining, we examined outcome indices that were prespecified for the interim (5-year) MTO follow-up (7).

We also examined a self-reported measure of comprehensive SWB—the first time the effect of neighborhoods on SWB has been assessed in an experimental analysis. Our primary measure of SWB is based on responses to the following question from the General Social Survey (GSS) that we included on our long-term follow-up survey of MTO adults: “Taken all together, how would you say things are these days—would you say that you are very happy, pretty happy, or not too happy?” (17). This type of happiness ques-

**Table 1.** Baseline characteristics (1994 to 1998) of adults interviewed as part of long-term survey ( $n = 3273$  interviewees), by randomized MTO treatment status. Mean values represent shares, except for age and income; missing values have been imputed (except income). Values are weighted to account for changes over time in treatment assignment likelihood and for the follow-up survey sampling design (supplementary materials, section 1). \*\*\* $P < 0.01$ , \*\* $P < 0.05$ , \* $P < 0.10$  on two-tailed  $t$  test of difference between MTO treatment and control groups.

	Control group mean	MTO treatment (voucher) groups mean
	$n = 1139$	$n = 2134$
Gender and age		
Female	0.978	0.984
Age as of 31 December 2007 (years)	44.5	44.6
Race and ethnicity		
African-American (any ethnicity)	0.660	0.640
Hispanic ethnicity (any race)	0.304	0.325
Other demographic characteristics		
Never married	0.637	0.623
Working	0.245	0.270
High school diploma	0.361	0.367
General Educational Development (GED) certificate	0.199	0.169*
Receiving Aid to Families with Dependent Children (AFDC)	0.763	0.752
Household characteristics		
Household income (2009 dollars)	\$12,438.64	\$12,833.64
Site		
Baltimore	0.135	0.136
Boston	0.205	0.203
Chicago	0.205	0.206
Los Angeles	0.226	0.225
New York	0.229	0.229
Neighborhood characteristics		
Household member was crime victim in past 6 months	0.416	0.425
Very dissatisfied with neighborhood	0.467	0.478
Primary or secondary reason for wanting to move		
To get away from gangs and drugs	0.779	0.770
Better schools for children	0.481	0.516*
To get a bigger or better apartment	0.457	0.440
To get a job	0.069	0.058

tion yields results similar to those from questions about general life satisfaction; both provide global retrospective assessments of how people think their lives are going and are increasingly used to assess public policy impacts (18). We used the same three-point response scale as the GSS to benchmark MTO against national samples; tradeoffs with this scaling are discussed in the supplementary materials. Another reason we focused on adults is because more is known about measuring SWB of adults than of youth (19). SWB was not included in the interim MTO survey but was added to the long-term survey to be one of the key summary measures of the net impacts on families from moving to a less distressed neighborhood. MTO controls are slightly happier than adults in national surveys with similar sociodemographic characteristics (table S2).

**Methods.** We conducted intention-to-treat (ITT) estimates that capture the effect of being

offered the chance to use an MTO voucher to move into a different neighborhood. These estimates were calculated as the difference in average outcomes for families assigned to treatment versus those assigned to the control condition. ITT estimation assumes that randomization was carried out correctly, that there is no selective attrition in measuring outcomes across groups, and that MTO's effect on a given family is independent of the treatment status of other families.

We also used the MTO experimental data to estimate the relationship between outcomes and some specific neighborhood attributes  $\mathbf{W}$ , as in Eq. 1. Ordinary least-squares estimation of Eq. 1 may yield biased estimates because of possible correlation of  $\mathbf{W}$  with unmeasured individual characteristics ( $\epsilon$ ) that influence both neighborhood selection and outcomes,  $Y$ . We instead used two-stage least-squares to generate instrumental variables (IV) estimates, where in the first stage of the equation we used interactions

of MTO random assignment and indicators for which MTO site families live in at baseline as instrumental variables to generate predicted values of  $\mathbf{W}$  that were then substituted for the actual value in the second stage Eq. 1 (7). The equation also includes a set of baseline characteristics,  $\mathbf{X}$ , including indicators for MTO demonstration site and numerous participant sociodemographic characteristics, to improve the precision of our estimates.

$$Y = \pi_0 + \mathbf{W}\pi_1 + \mathbf{X}\pi_2 + \epsilon \quad (1)$$

IV estimation of Eq. 1 essentially fits a “dose-response” model and asks whether those treatment groups and sites that experience relatively larger gains in specific elements of  $\mathbf{W}$  as a result of treatment assignment also experience relatively larger gains in the outcome of interest. This estimation approach assumes that this is the only reason why the effect of treatment assignment on outcomes varies across randomized groups and demonstration sites. It also assumes that the only pathway through which the instruments affect the outcomes of interest is by affecting the neighborhood measures included in Eq. 1. Given the large number of neighborhood attributes affected by MTO moves, this approach cannot isolate the effect of a specific attribute. We instead view any single variable used in  $\mathbf{W}$  to be a summary measure of neighborhood environment (for example, tract poverty captures the effects of moving to an area with a lower poverty rate and other aspects of neighborhood economic disadvantage that co-vary with tract poverty).

In a model that relates  $Y$  to a single neighborhood measure  $\mathbf{W}$  with the only covariates ( $\mathbf{X}$ ) being the indicators for the MTO cities, the IV estimation of Eq. 1 is equivalent to fitting a regression line through the 15 data points that correspond to the average values of  $Y$  and  $\mathbf{W}$  for each of the three randomized MTO groups in the five demonstration sites relative to the site overall mean. Below, we present several visual instrumental variables graphs that show the data and logic behind our IV estimates.

**Results.** As shown in Table 2, MTO does indeed generate sizable and sustained differences in average neighborhood conditions of the individuals across randomly assigned groups, despite the fact that only around half the adults assigned to treatment used a MTO voucher to relocate. One year after random assignment, the average control group family is living in a census tract with a poverty rate of 50%, compared with 34% for the average family assigned to treatment (SE of the difference  $\pm 0.7\%$ ). This difference in tract poverty across randomized groups narrowed over time, mostly because tract poverty rates declined for controls over time. This decline was driven by control families increasingly moving into lower-poverty neighborhoods on their own, as opposed to their baseline neighborhoods gentrifying around them. Averaged over the entire study period, assignment

**Table 2.** MTO effects on post-randomization housing and neighborhood conditions of adult participants interviewed in long-term survey. Table shows average outcomes for control group adults and ITT contrast of outcomes for adults assigned to treatment (pooling the low-poverty and traditional voucher groups) rather than control. Housing and neighborhood conditions were measured from long-term survey data and census tract-level data interpolated from the 1990 and 2000 decennial censuses and the 2005–2009 American Community Survey. ITT was calculated by using ordinary least-squares regression controlling for baseline covariates, using weights (Table 1 and supplementary materials, sections 1 and 5). \*\*\* $P < 0.01$ , \*\* $P < 0.05$ , \* $P < 0.10$  on two-tailed  $t$  test.

	Control mean	MTO treatment (voucher) groups versus control			
		ITT		SE	<i>n</i>
Census tract characteristics					
Share poor at different points in time					
1 year after random assignment	0.499	−0.160	***	(0.007)	3224
5 years after random assignment	0.399	−0.089	***	(0.007)	3208
10 to 15 years after random assignment (May 2008)	0.311	−0.034	***	(0.007)	3206
Share poor for all addresses since random assignment (duration-weighted)					
Share poor	0.396	−0.082	***	(0.005)	3270
Share poor, z score using U.S. tract poverty distribution	2.082	−0.666	***	(0.041)	3270
Share poor, z score using MTO control group tract poverty distribution	0.000	−0.653	***	(0.040)	3270
Duration-weighted poverty rate is...					
Less than 20%	0.054	0.196	***	(0.013)	3270
Less than 30%	0.242	0.237	***	(0.018)	3270
Less than 40%	0.512	0.206	***	(0.018)	3270
Share minority					
10 to 15 years after random assignment (May 2008)	0.844	−0.024	**	(0.009)	3206
All addresses since random assignment (duration-weighted)	0.880	−0.046	***	(0.006)	3270
Residential mobility					
Number of moves after random assignment	2.165	0.584	***	(0.068)	3273
Self-reports on long-term (10- to 15-year) follow-up surveys about neighborhood and housing conditions					
Feel unsafe during day	0.196	−0.039	**	(0.015)	3262
Number of housing problems (0 to 7)	2.051	−0.380	***	(0.076)	3267
Likely or very likely to report kids spraying graffiti (collective efficacy)	0.589	0.064	***	(0.020)	3255
One or more friends with college degree	0.532	0.049	**	(0.020)	3203



to treatment reduced average tract poverty rates by 8.2 percentage points ( $SE \pm 0.5\%$ ), or about one fifth of the control group average of 40%. This is equal to about two thirds of a SD reduction in tract poverty in the national tract-poverty distribution.

MTO had more modest effects on neighborhood racial composition, as shown in Table 2. Assignment to treatment reduced the average neighborhood minority share experienced by participants over the study period by 4.6 percentage points ( $SE \pm 0.6\%$ ), a small share of the control group's average of 88%, although there are larger treatment-control differences in this variable in some sites than others (this is the source of variation we used for our instrumental variables estimates; supplementary materials, section 3.3). Table 2 further indicates MTO generated sustained effects on neighborhood safety and other neighborhood social processes, such as collective efficacy that are thought to be important in changing behavior (3, 5).

Because moving itself is part of the MTO treatment and could have independent effects on people's life outcomes, it is important to keep in mind that the control group averaged 2.165 moves over the study period (Table 2). Treatment assignment increased the number of moves over 10 to 15 years by 0.584 ( $SE \pm 0.068$ ).

As shown in Fig. 1, the opportunity to move through MTO had mixed (null to positive) long-term effects on objective measures of well-being of the type that have been the traditional focus of the neighborhood effects literature. ITT effects are not statistically significant on economic outcomes for adults in MTO households 10 to 15 years after random assignment. Effects on a broad index of physical health measures are in the direction of better health (ITT effect of  $+0.060$  SDs,  $SE \pm 0.039$ ) but are not quite statistically significant ( $P = 0.12$ ; unless otherwise noted, all remaining statistical results come from *t* tests). Effects on mental health are marginally significant ( $P = 0.084$ ) in the direction of better health (ITT effect of  $+0.070$  SDs,  $SE \pm 0.041$ ). However, ITT effects are more strongly beneficial for SWB (Fig. 1, far right bar), with the offer to move to a less disadvantaged area increasing SWB by  $+0.098$  SDs ( $SE \pm 0.039$ ,  $P = 0.013$ ).

The basic intuition behind our instrumental variables estimates, which try to distinguish between the effects on SWB of neighborhood economic disadvantage (as represented by tract poverty rate) versus racial segregation (as measured by tract share minority), are shown in Fig. 2. The *x* axis of Fig. 2A represents the average tract poverty rate MTO adults experience over the study period, whereas the *y* axis represents SWB, both in standardized (*z* score) form. The data points are the average tract poverty and SWB for adults broken out by MTO randomized group and demonstration site. The slope of this line is essentially our IV estimate of the relationship between SWB and tract poverty. A 1-SD decrease in tract poverty (a 13-percentage-point change) is

associated with an increased SWB equal to 0.141 SDs ( $SE \pm 0.054$ ,  $P = 0.0009$ ) (table S5).

As suggested by Fig. 2, B to D, poverty concentration is more important than is racial segregation in affecting the SWB of MTO adults. SWB does not have a statistically significant relationship with the minority composition of the tracts in which MTO families reside ( $P = 0.478$ ), as illustrated by the relatively flat line in Fig. 2B. The size of the increase in SWB from a 1-SD reduction in tract poverty nearly doubles once we control for tract minority share in the same model (from 0.141 to 0.261 SDs,  $SE \pm 0.093$ ,  $P = 0.005$ ) (table S9), as seen by comparing Fig. 2, A and C. In contrast, holding neighborhood poverty constant, a 1-SD decrease in neighborhood minority share makes MTO adults, if anything, worse off ( $-0.279$  SDs,  $SE \pm 0.169$ ,  $P = 0.098$ ), as shown by the positive slope in Fig. 2D. The conclusion that a decline in neighborhood economic disadvantage has a more beneficial result for SWB than does a comparably sized decline in neighborhood minority composition comes from the fact that we can reject the null hypothesis that the slopes illustrated by Fig. 2, C and D, are equal ( $P = 0.030$ ) (table S9).

Results are qualitatively similar if we estimate models that assume that outcomes are only affected by current neighborhood conditions, measured at the start of the survey period, May 2008 (tables S6 and S10 and figs. S4 to S7).

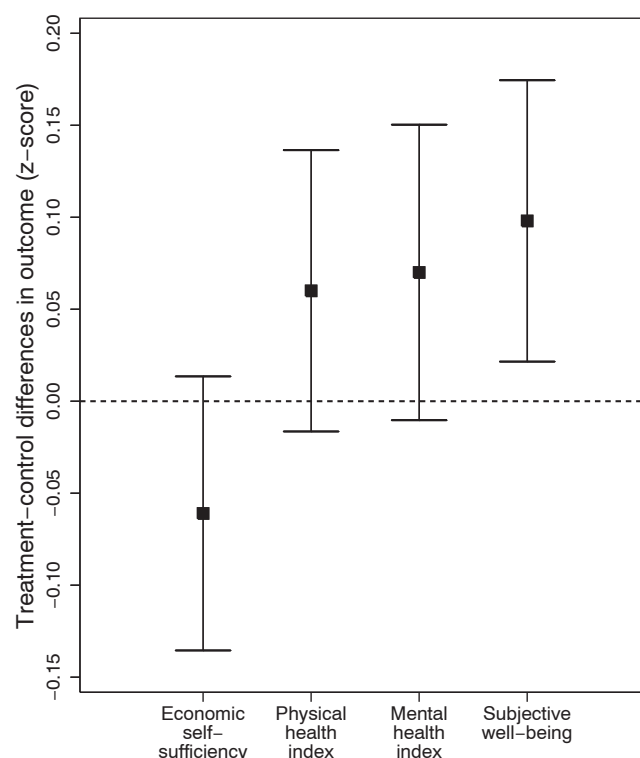
**Discussion.** To what extent does moving to a less distressed neighborhood environment affect people's well-being? In this Research Article, we present results from a large-scale randomized social experiment (MTO) designed to address this

question, which has been of long-standing concern to the social and medical sciences and to policy-makers. Random assignment in MTO overcomes concerns with selection bias by generating differences in the average neighborhood conditions experienced by otherwise comparable groups of people. MTO is unique in terms of the long duration of the follow-up data collection that has been carried out with participants spanning 10 to 15 years after randomization.

MTO has strong internal validity, but the MTO findings may not generalize to all U.S. families. Although the MTO sample is comparable with other urban minority samples in high-poverty urban areas that have been studied in this literature (20, 21), the sorts of families living in such extreme-poverty areas are very disadvantaged relative to other American adults. MTO was carried out during a time when concentrated poverty and crime rates were declining, and HUD's HOPE VI program was demolishing many public housing projects across the country. MTO's impacts also do not necessarily identify the effects of larger-scale mobility programs (22).

Keeping these caveats in mind, we find that over the long term (10 to 15 years) the chance to move to less distressed neighborhoods in MTO has no detectable long-term effects on adult economic self-sufficiency. In a previous paper, we showed that MTO had important long-term effects on two particularly important physical health measures that predict long-term disease risk; namely, extreme obesity and diabetes (15). We report here that MTO's impact on a broader index of physical health was in the same direction (toward improved health) but was not quite statistically

**Fig. 1.** Impact on each outcome of assignment to the MTO treatment (voucher) groups for adults interviewed in a long-term survey. The squares represent the ITT estimate for the effect of being assigned to MTO treatment (pooling low-poverty and traditional voucher groups), rather than control, for the outcomes listed on the *x* axis: economic self-sufficiency, physical health, mental health, and SWB (Table 2 and supplementary materials, sections 1, 4, and 5). The box whiskers represent the 95th percent confidence interval around the estimates.



significant, whereas we found a marginally significant beneficial impact of moving to a less distressed neighborhood on a broad index of mental health.

This mixed pattern of MTO impacts for traditional, objective measures of well-being echo

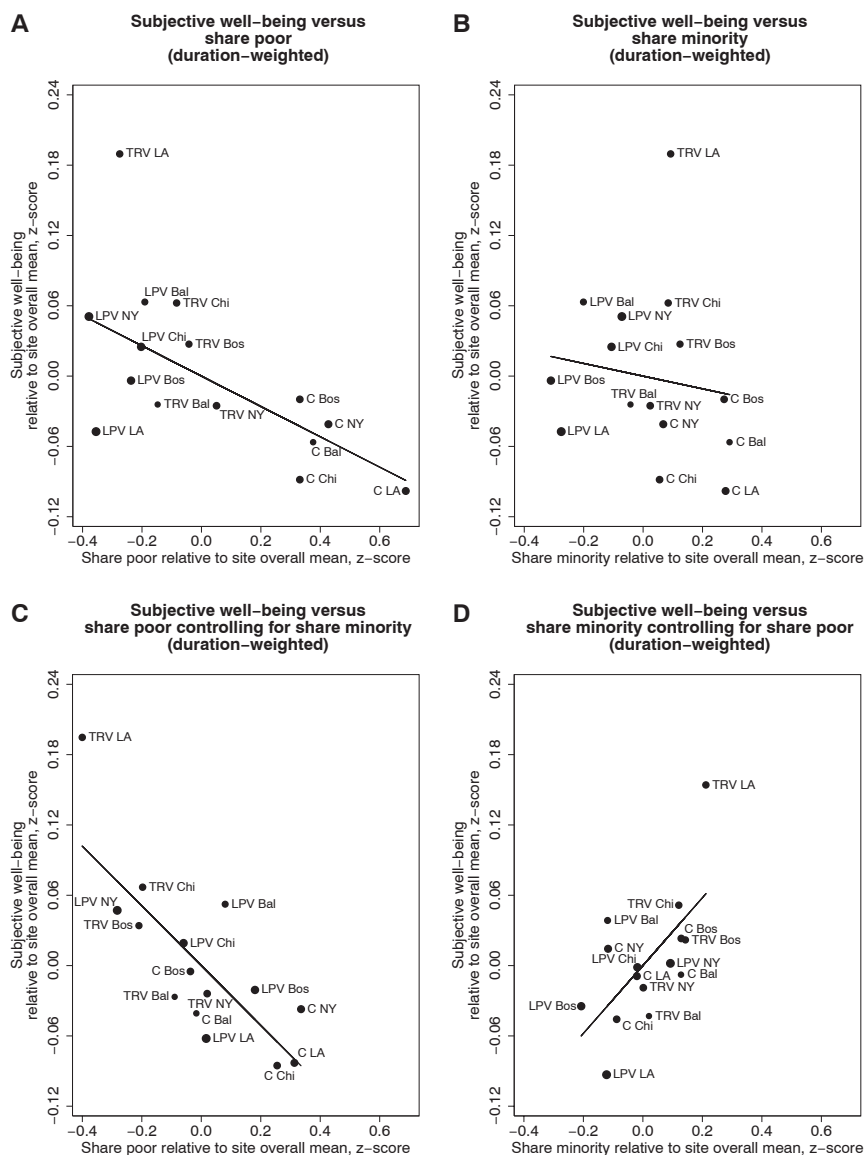
what was found in the interim (5-year) follow up of MTO families (7, 23). These mixed results have been disappointing to many observers, in part because the congressional legislation authorizing the MTO demonstration explicitly mentioned the goal of improving some outcomes that were

unaffected (such as adult earnings). Similar mixed findings are apparent in recent quasi-experimental studies of other housing mobility programs (24–26). These mixed results have led influential observers such as Yale Law School professor Robert Ellickson, who is generally sympathetic to the value of housing vouchers over project-based housing programs, to argue that “recently published studies have begun to destabilize the former consensus that a poor adult or child is significantly disadvantaged by residing among other poor people ... the case for dismantling an entire poor neighborhood ... is hardly so plain” [(27), p. 439].

Yet, the results reported here might lead to quite a different conclusion, in that we find sizable positive effects of moving from a more distressed to a less distressed neighborhood on SWB, a measure that represents a comprehensive assessment by the participants themselves of the extent to which their lives have been affected. Our results suggest that living in distressed neighborhoods has more important adverse impacts, and escaping from such neighborhoods has more important positive effects, on the well-being of low-income adults than was revealed by previous experimental and quasi-experimental studies of neighborhood effects that focused on traditional measures of socioeconomic and health outcomes. Whether or not the MTO vouchers imposed additional locational constraints on families does not appear to matter much for the positive effects of such moves on well-being (table S4).

Although “happiness” has no natural metric, one can still interpret the magnitude of our results by noting that a 1-SD reduction in neighborhood poverty (about 13 percentage points) is associated with an increase in SWB that is about two-thirds of the gap in SWB between U.S. blacks and whites [which is around one quarter of a SD in favor of whites (28)] and about equal to the remaining gap in SWB between families with annual incomes that differ by \$13,000 after conditioning on a standard set of control variables that differ by income and affect happiness (supplementary materials, section 3.3). This is a large amount, equal to about two thirds of the average income of MTO control group families in our long-term survey (\$20,000).

Subject self-reports of SWB have the potential to provide an informative summary measure of the overall impact of neighborhood conditions on people's lives. Although SWB measures are being used with increased frequency in the social sciences and policy analysis, SWB has not been the focus of much previous “neighborhood effects” research. The proper interpretation of self-reports about SWB remains the topic of some debate. Previous studies show different measures of self-reported SWB to be correlated in expected ways with objective indicators of well-being such as life events, biological indicators (such as smiling frequency and brain activity), and reports from significant others about the person's happiness at



**Fig. 2.** Instrumental variable estimation of the relationship between SWB and average (duration-weighted) (A) tract poverty rate, (B) tract share minority, (C) tract poverty controlling for minority share, and (D) tract minority share controlling for tract poverty. The y axis is a three-point happiness scale (1 = not too happy, 2 = pretty happy, 3 = very happy) expressed in SD units relative to the control group. Share poor is the fraction of census tract residents living below the poverty threshold. Share minority is the fraction of census tract residents who are members of racial or ethnic minority groups. Tract shares are linearly interpolated from the 1990 and 2000 decennial census and 2005 to 2009 American Community Survey and are weighted by the time respondents lived at each of their addresses from random assignment through May 2008. Share poor and minority are z scores, standardized by the control group mean and SD. The points represent the site (Bal, Baltimore; Bos, Boston; Chi, Chicago; LA, Los Angeles; NY, New York City) and treatment group (LPV, low-poverty voucher; TRV, traditional voucher; C, control group). The slope of the line is equivalent to a two-stage least-squares estimate of the relationship between SWB and the mediator shown in each panel, using interactions of indicators for MTO treatment group assignment and demonstration site as instruments for the mediator (controlling for site indicator main effects).

both the individual and group levels (29, 30) (supplementary materials, section 2.3). We also corroborate our findings for SWB by examining the effects of MTO moves on related measures of psychological distress (table S4).

As noted in the introduction, it is also important for both science and policy to understand why changes in neighborhood environments affect the well-being of low-income adults. Isolating mechanisms with the MTO data are challenging, and our statistical power to do so is somewhat limited. We focused on distinguishing the effects of residential income segregation versus racial segregation because this is a key scientific question, because different policies may be required to address segregation by income versus race, and because racial segregation has declined the past 40 years whereas income segregation has substantially increased.

Our results suggest that changes in neighborhood poverty are more important than racial segregation in affecting the SWB of low-income adults in MTO. (We interpret neighborhood poverty as a marker for a collection of correlated neighborhood characteristics across the neighborhoods in which the MTO families reside.) The same qualitative pattern holds for adult physical and mental health outcomes as well (supplementary materials).

The rise in U.S. residential income segregation since 1970 raises the possibility that the problem of harmful neighborhood effects on people's well-being may be getting worse rather than better over time. Increased poverty concentration in America does not seem to be simply due to increases in overall income inequality (31). The average tract poverty rate for families in the bottom quintile of the U.S. income distribution increased over the past 40 years by about 2.4 percentage points (from 17.6 to 20.0%). If the results from our MTO sample generalize to other very low-income families, the increase in poverty concentration over the past 40 years reduced the well-being of the bottom quintile of the income distribution by an amount that may be equivalent to a decline in annual household income of about \$1400 (~8%). If our estimates are correct, the \$1400 dollar-equivalent for the decline in well-being for families in the bottom quintile caused by increased poverty concentration from 1970 to 2007 is about equal in size to the total gain in real annual family income of \$1300 that the bottom quintile has experienced over roughly the past 40 years from \$15,336 in 1969 to \$16,622 in 2007 [(32), converted to 2009 dollars; supplementary materials, section 3.3].

Our findings are also germane to debates about the proper objectives for public policy. For example, one recent review of U.S. antipoverty programs notes that their effectiveness depends "at least in part, on whether the programs do, in fact, reduce poverty" [(33), p. 12]. By that standard, MTO-type policy efforts to improve the neighborhood conditions of poor families would not be part of an effective antipoverty strategy

because the program failed to produce detectable impacts on family income (7, 23). But if the goal is the broader one of improving the well-being of poor families, then policies that seek to ameliorate the adverse effects of dangerous, distressed neighborhoods on poor families are worthy of careful consideration.

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## Supplementary Materials

[www.sciencemag.org/cgi/content/full/337/6101/1505/DC1](http://www.sciencemag.org/cgi/content/full/337/6101/1505/DC1)  
Materials and Methods  
Supplementary Text  
Figs. S1 to S7  
Tables S1 to S10  
References (34–95)

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# Kepler-47: A Transiting Circumbinary Multiplanet System

Jerome A. Orosz,<sup>1\*</sup> William F. Welsh,<sup>1</sup> Joshua A. Carter,<sup>2</sup> Daniel C. Fabrycky,<sup>3</sup> William D. Cochran,<sup>4</sup> Michael Endl,<sup>4</sup> Eric B. Ford,<sup>5</sup> Nader Haghighipour,<sup>6</sup> Phillip J. MacQueen,<sup>4</sup> Tsevi Mazeh,<sup>7</sup> Roberto Sanchis-Ojeda,<sup>8</sup> Donald R. Short,<sup>1</sup> Guillermo Torres,<sup>2</sup> Eric Agol,<sup>9</sup> Lars A. Buchhave,<sup>10,11</sup> Laurance R. Doyle,<sup>12</sup> Howard Isaacson,<sup>13</sup> Jack J. Lissauer,<sup>14</sup> Geoffrey W. Marcy,<sup>13</sup> Avi Shporer,<sup>15,16,17</sup> Gur Windmiller,<sup>1</sup> Thomas Barclay,<sup>14,18</sup> Alan P. Boss,<sup>19</sup> Bruce D. Clarke,<sup>12,14</sup> Jonathan Fortney,<sup>3</sup> John C. Geary,<sup>2</sup> Matthew J. Holman,<sup>2</sup> Daniel Huber,<sup>14</sup> Jon M. Jenkins,<sup>12,14</sup> Karen Kinemuchi,<sup>14,18</sup> Ethan Kruse,<sup>2</sup> Darin Ragozzine,<sup>2</sup> Dimitar Sasselov,<sup>2</sup> Martin Still,<sup>14,18</sup> Peter Tenenbaum,<sup>12,14</sup> Kamal Uddin,<sup>14,20</sup> Joshua N. Winn,<sup>8</sup> David G. Koch,<sup>14</sup> William J. Borucki<sup>14</sup>

We report the detection of Kepler-47, a system consisting of two planets orbiting around an eclipsing pair of stars. The inner and outer planets have radii 3.0 and 4.6 times that of Earth, respectively. The binary star consists of a Sun-like star and a companion roughly one-third its size, orbiting each other every 7.45 days. With an orbital period of 49.5 days, 18 transits of the inner planet have been observed, allowing a detailed characterization of its orbit and those of the stars. The outer planet's orbital period is 303.2 days, and although the planet is not Earth-like, it resides within the classical "habitable zone," where liquid water could exist on an Earth-like planet. With its two known planets, Kepler-47 establishes that close binary stars can host complete planetary systems.

The extremely precise and nearly continuous observations provided by the Kepler spacecraft (1) has enabled the detection of more than 2300 planet candidates (2, 3) and over 2100 eclipsing binary stars (4, 5). A synergy of

these efforts has helped establish the class of circumbinary planets, which are planets that orbit around a pair of stars (6–8). Their detection has led to a revitalized effort to understand planet formation around binary stars (9, 10). A circumbinary planet can reveal itself in two ways. If the planet's orbital plane is favorably aligned, the planet may transit across one or both of the stars, causing a small decrease in the amount of light from the system. If the planet is sufficiently massive and close, the planet can perturb the stellar orbits (11). The most readily observable manifestation of this perturbation is a change in the times when the eclipses occur.

In contrast to a single planet orbiting a single star, a planet in a circumbinary system must transit a "moving target." Consequently, the time intervals between the transits, as well as their duration, can vary substantially. The transits can deviate from having a constant period by up to several days and can vary in duration by several hours. These transit timing and duration variations can be taken to be the signature of a circumbinary planet because no other known mechanism can cause such effects. Modeling of the timing and duration changes can be used to precisely determine the orbits of the planet and stars (6–8).

Kepler observations of the binary star system Kepler-47 (KIC 10020423, also KOI-3154) show primary eclipses (smaller and fainter star blocking the brighter, more massive "primary" star) every 7.45 days with a depth of 13%. Also present are secondary eclipses with a depth of 0.8% (Fig. 1) and a quasiperiodic modulation in the out-of-eclipse regions of ~2 to 4% caused by star-spots on the primary star (12). The Kepler data span

1050.5 days, and visual inspections of the light curve revealed the signals of two candidate circumbinary planets, with periods of ~50 and ~303 days. Three transits of the longer-period candidate (hereafter the outer planet) were readily apparent, but those of the shorter-period candidate (hereafter the inner planet) were more difficult to find because of their shallower depth. Using the predictions of a preliminary model of the system as a guide, we detected a total of 18 transits of the inner planet. The transits have timings that can deviate from strict periodicity by up to several hours and their durations vary significantly, strongly suggesting that they originate from circumbinary planets (Fig. 2). All of these events are transits over the primary star.

To characterize the stellar orbit, we obtained Doppler spectroscopy of the system. Radial-velocity variations of the primary star were readily detected, but the secondary star is too faint to have been measured. Usually when the radial-velocity measurements of only one component in a spectroscopic binary are available, the masses of the stars cannot be uniquely determined. However, the transit times and durations provide constraints on the geometric configuration of the stellar orbits and specify the stellar mass ratio, which in combination with the primary's radial velocities, allow both stellar masses and the physical scale to be determined.

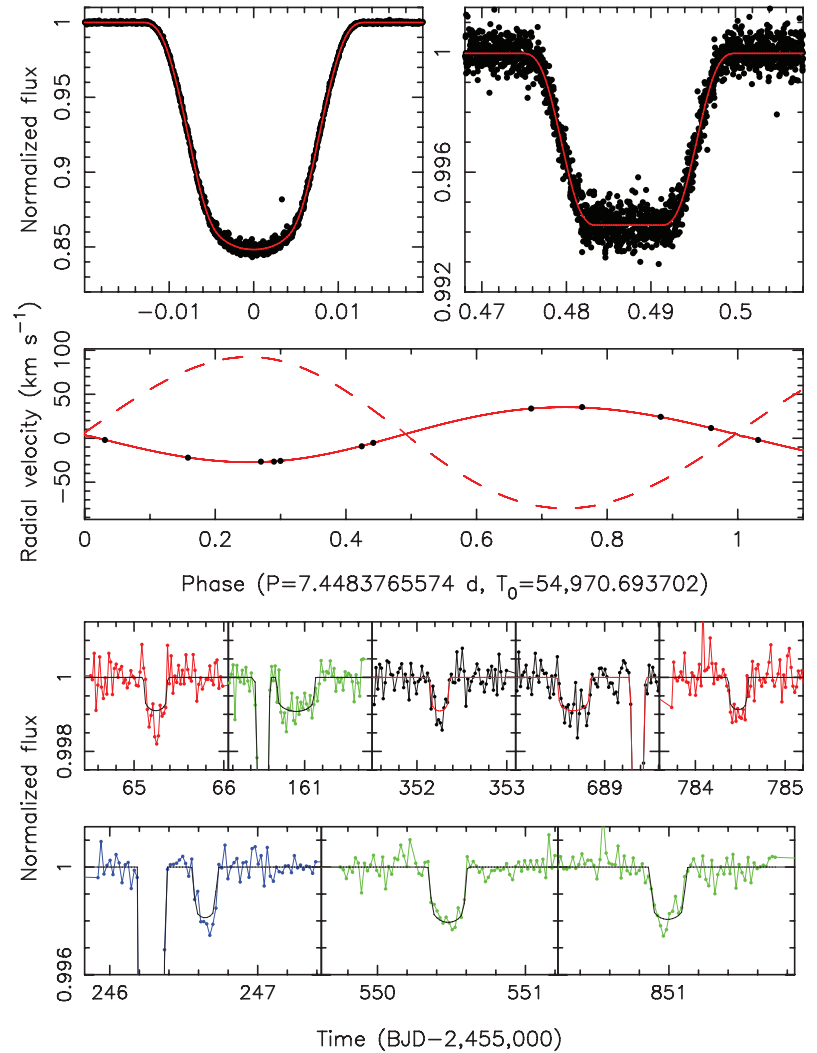
To determine the system parameters, we used a photometric-dynamical model (13) similar to that used for the four previously known transiting circumbinary planets (6–8). This model assumes spherical bodies interacting via Newtonian gravity (12) and is used to fit the radial-velocity data and the Kepler time-series photometry. We determined the stellar masses as described above, and the relative sizes of the bodies from the eclipses and transits in the light curve. Information on the inclination, eccentricity, and mutual inclination of the planetary orbits is also implicit in the combination of photometric and radial-velocity data. Gravitational perturbations caused by the planets on the stars and on each other could, in principle, also constrain the masses, but for Kepler-47 the expected masses of the planets are too small to create a measurable effect over the time span of our data. The small radii of the transiting objects strongly suggest that they are of planetary mass (Table 1); dynamical considerations described below make this conclusion secure.

The inner planet, Kepler-47 b, is the smallest transiting circumbinary planet yet detected, with a radius of  $3.0 \pm 0.1$  Earth radii. Its mass is too small to be directly measured, but a  $3\sigma$  upper limit of 2 Jupiter masses has been determined based on the nondetection of timing variations of the stellar orbit (12). Because the planet's mass is unknown, its density is also unknown, and it is not possible to distinguish between a rocky composition and a more volatile-enriched composition. We can make a plausible mass estimate by

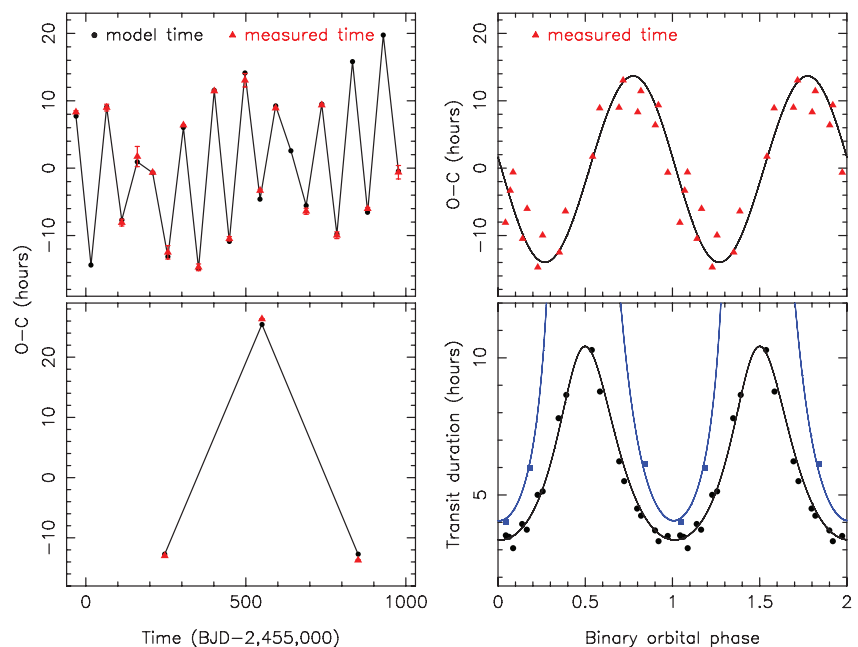
<sup>1</sup>Astronomy Department, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA. <sup>2</sup>Harvard-Smithsonian Center for Astrophysics, 60 Garden Street, Cambridge, MA 02138, USA. <sup>3</sup>Department of Astronomy and Astrophysics, University of California, Santa Cruz, CA 95064, USA. <sup>4</sup>McDonald Observatory, The University of Texas at Austin, Austin, TX 78712-0259, USA. <sup>5</sup>Astronomy Department, University of Florida, 211 Bryant Space Sciences Center, Gainesville, FL 32611, USA. <sup>6</sup>Institute for Astronomy and NASA Astrobiology Institute University of Hawaii-Manoa, 2680 Woodlawn Drive, Honolulu, HI 96822, USA. <sup>7</sup>School of Physics and Astronomy, Tel Aviv University, Tel Aviv 69978, Israel. <sup>8</sup>Department of Physics, and Kavli Institute for Astrophysics and Space Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. <sup>9</sup>Department of Astronomy, BOX 351580, University of Washington, Seattle, WA 98195, USA. <sup>10</sup>Niels Bohr Institute, University of Copenhagen, Juliane Maries vej 30, 2100 Copenhagen, Denmark. <sup>11</sup>Centre for Star and Planet Formation, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark. <sup>12</sup>SETI Institute, 189 Bernardo Avenue, Mountain View, CA 94043, USA. <sup>13</sup>Astronomy Department, University of California, Berkeley, CA 94720, USA. <sup>14</sup>NASA Ames Research Center, Moffett Field, CA 94035, USA. <sup>15</sup>Las Cumbres Observatory Global Telescope Network, 6740 Cortona Drive, Suite 102, Santa Barbara, CA 93117, USA. <sup>16</sup>Department of Physics, Broida Hall, University of California, Santa Barbara, CA 93106, USA. <sup>17</sup>Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA. <sup>18</sup>Bay Area Environmental Research Institute, Inc., 560 Third Street West, Sonoma, CA 95476, USA. <sup>19</sup>Department of Terrestrial Magnetism, Carnegie Institution for Science, 5241 Broad Branch Road NW, Washington, DC 20015-1305, USA. <sup>20</sup>Orbital Sciences Corporation, 45101 Warp Drive, Dulles, VA 20166, USA.

\*To whom correspondence should be addressed. E-mail: orosz@sciences.sdsu.edu

**Fig. 1.** Light curves and velocity curve data with model fits. **(Top)** Normalized and detrended flux is plotted versus orbital phase for the primary (left) and secondary (right) eclipses, along with the binary star model. **(Middle)** The radial velocities of the primary star and the best-fitting model are plotted versus the orbital phase. The expected radial-velocity curve of the secondary star is shown with the dashed line. **(Bottom)** The normalized and detrended flux near five representative transits of the inner planet (upper) and all three transits of the outer planet (lower) are shown. See figs. S13, S14, and S15 for plots of all 18 transits of the inner planet and plots of the residuals of the various model fits.



**Fig. 2.** Planetary transit time and duration variations. **(Left)** The observed minus expected times of transit computed from a linear ephemeris are shown versus time (an “O-C” curve). The triangles show the measured deviations, and the filled circles are the predictions from the photometric-dynamical model. Four transits of the inner planet occurred in data gaps or regions of corrupted data. **(Top right)** The O-C values of the inner planet are shown as a function of the binary phase, where the primary eclipse occurs at phase 0.0 and the secondary eclipse is at phase 0.487. Two cycles have been shown for clarity. The solid curve is the predicted deviation assuming a circular, edge-on orbit for the planet. The lateral displacement of the primary near the eclipse phases is minimal, and therefore the deviation of the transit time from a linear ephemeris is near zero. The primary is maximally displaced near the quadrature phases, so transits near those phases show the most offset in time. **(Bottom right)** The durations of the transits for the inner planet (filled circles) and the outer planet (filled squares) as a function of the orbital phase of the binary. The solid curves are the predicted durations assuming a circular, edge-on orbit for the planet. At phases near the primary eclipse, the planet and the primary star are moving in opposite directions, resulting in a narrower transit. At phases near the secondary eclipse, the planet and the primary star are moving in the same direction, resulting in a longer transit. The outer planet is moving slower than the inner planet, resulting in longer transits at the same binary phase.



using both an empirical mass-radius relation based on transiting exoplanets (14) and a limited empirical mass-radius relation for planets in the solar system (15), yielding ~7 to 10 Earth masses

or ~0.4 to 0.6 Neptune masses. The planet's 49.5-day orbital period is 6.6 times the period of the stellar binary. This is ~77% longer than the critical period (28 days) within which the planet

would be susceptible to dynamical instability due to interactions with the stars (16). Although this 77% margin is notably larger than for the other known transiting circumbinary planets—i.e., 14, 21, 24, and 42% for Kepler 16, 34, 35, and 38, respectively—the planet is still somewhat close to the instability limit, a feature shared by all known transiting circumbinary planet systems.

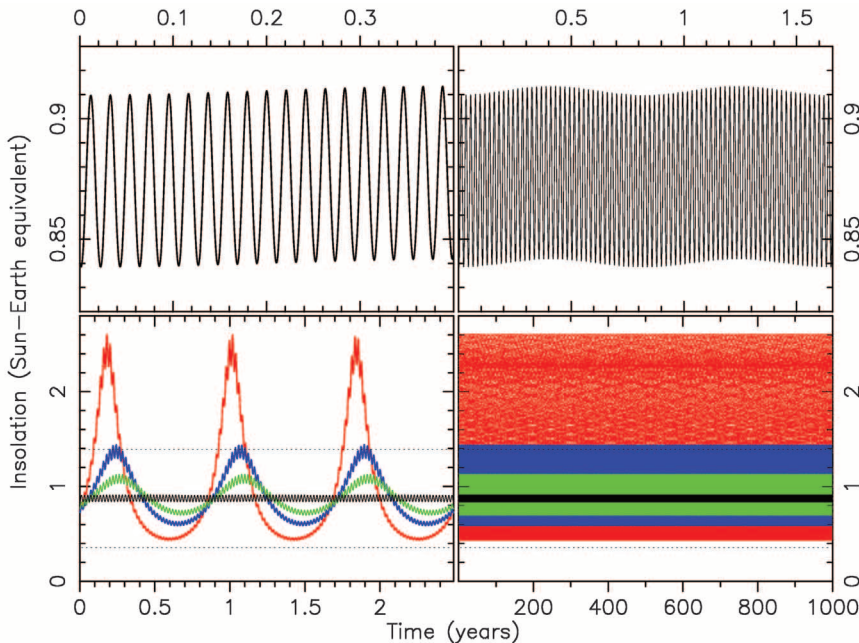
The outer planet, Kepler-47 c, has a radius of  $4.6 \pm 0.2$  Earth radii, making it slightly larger than the planet Uranus. As before, the planet's mass is too small to be measured directly, and we derived a  $3\sigma$  upper limit of 28 Jupiter masses (12). Based on its radius, we find a plausible mass of ~16 to 23 Earth masses or ~0.9 to 1.4 Neptune masses, using these empirical mass-radius relations (14, 15). With only three transits currently available, the outer planet's orbital eccentricity is poorly constrained. A perfectly circular orbit would fit the data, and a low-eccentricity orbit seems plausible given the low eccentricity of the stellar binary ( $e = 0.023$ ) and of planet b ( $e < 0.035$ ). The photometric-dynamical model provides only an upper limit on the eccentricity,  $e < 0.4$  with 95% confidence, and the requirement of long-term stability only rules out eccentricities larger than 0.6 (12).

Owing to the orbital motion of the stars, the outer planet is subject to variations in the incident stellar flux (i.e., insolation), even if the planet's orbit is circular (Fig. 3). The average insolation is similar to the amount that Earth receives from the Sun; for a circular orbit, it is 87.5% of the Sun-Earth insolation and varies by ~9%. This places Kepler-47 c well within the classical "habitable zone," defined as the range of distances from the host star(s) where liquid water could persist on the surface of an Earth-like planet (17). While Kepler-47 c is probably a gas giant and thus not suitable for life, its location is notable as it

**Table 1.** A summary of the results for the photometric-dynamical model. For brevity, some of the fitting parameters are not listed here. See table S5 for a complete listing of fitting parameters. AU, astronomical units.

Parameter	Best fit	1 $\sigma$ uncertainty
<i>Bulk properties</i>		
Mass of star A, $M_A$ ( $M_\odot$ )	1.043	0.055
Mass of star B, $M_B$ ( $M_\odot$ )	0.362	0.013
Radius of star A, $R_A$ ( $R_\odot$ )	0.964	0.017
Radius of star B, $R_B$ ( $R_\odot$ )	0.3506	0.0063
Temperature of star A, $T_{\text{eff},A}$ (K)	5636	100
Temperature of star B, $T_{\text{eff},B}$ (K)	3357	100
Luminosity of star A, $L_A$ ( $L_\odot$ )	0.840	0.067
Luminosity of star B, $L_B$ ( $L_\odot$ )	0.014	0.002
Radius of planet b, $R_b$ ( $R_\oplus$ )	2.98	0.12
Radius of planet c, $R_c$ ( $R_\oplus$ )	4.61	0.20
<i>Stellar orbit</i>		
Semimajor axis, $a_{AB}$ (AU)	0.0836	0.0014
Orbital period, $P_{AB}$ (day)	7.44837695	0.00000021
Eccentricity, $e_{AB}$	0.0234	0.0010
Argument of periaapse, $\omega_{AB}$ (degrees)	212.3	4.4
Orbital inclination, $i_1$ (degrees)	89.34	0.12
<i>Planet b orbit</i>		
Semimajor axis, $a_b$ (AU)	0.2956	0.0047
Orbital period, $P_b$ (day)	49.514	0.040
Eccentricity (95% conf.), $e_b$	<0.035	
Orbital inclination, $i_b$ (degrees)	89.59	0.50
Mutual orbital inclination, $I_b$ (degrees)	0.27	0.24
<i>Planet c orbit</i>		
Semimajor axis, $a_c$ (AU)	0.989	0.016
Orbital period, $P_c$ (day)	303.158	0.072
Eccentricity (95% conf.), $e_c$	<0.411	
Orbital inclination, $i_c$ (degrees)	89.826	0.010
Mutual orbital inclination, $I_c$ (degrees)	1.16	0.46

**Fig. 3.** The time-varying insolation  $S$  received by Kepler-47 c, for different assumed eccentricities. The insolation is in units of the solar luminosity at a distance of 1 AU ( $S_{\text{Sun}} = 1368 \text{ W m}^{-2}$ ). The upper panels are for a zero eccentricity orbit and highlight the insolation variations caused by the 7.4-day orbit of the binary. The lower panels show eccentricities of  $e = 0.0, 0.1, 0.2$ , and  $0.4$  (colored black, green, blue, and red, respectively) and illustrate the longer time-scale variations. The dotted lines mark the limits for the inner and outer edges of the habitable zone, following the prescription in (24) for the onset of a runaway greenhouse effect and the maximum greenhouse effect.





demonstrates that circumbinary planets can exist in habitable zones. Although the definition of the habitable zone assumes a terrestrial planet atmosphere, which does not apply for Kepler-47 c, large moons, if present, would be interesting worlds to investigate.

A 0.2% deep transit-like event is present at time 2,455,977.363 (BJD, barycentric Julian date) that is not caused by either of the two planets. A search for additional transits has revealed several more tentative transit events (12), but we caution that the star is faint (the Kepler magnitude is 15.178), there are large modulations due to star-spots, and the data contain correlated “red” noise, making small, nonperiodic transit detection challenging. The marginal evidence at present is insufficient to place confidence on any additional candidate planet(s).

The primary star is similar to the Sun in both mass and radius, and dominates the luminosity of the binary system, having 60 times the bolometric luminosity of the secondary star (or 176 times the brightness in the Kepler bandpass). A spectroscopic analysis gives an effective temperature of  $5636 \pm 100$  K for the primary star (table S2), with a metallicity slightly less than solar ( $[M/H] = -0.25 \pm 0.08$  dex). The star’s rotation period as determined from the star-spot modulation in the light curve (12) is only 4% longer than the orbital period, suggesting that the spin and orbital angular momenta have been synchronized by tidal interactions. Supporting this interpretation, the obliquity of the primary star (the angle between the spin and orbital axes) must be smaller than about  $20^\circ$ , based on the observable effects of the secondary star eclipsing star-spots on the primary star (12, 18–21). Star-spot crossings also perturb the shape and depth of the primary eclipses, leading to systematic trends in the eclipse times, and limit the precision with which one can infer the planets’ masses. In addition, the loss of light due to star-spots causes eclipses to appear slightly deeper than they would for an unspotted star, biasing the determination of the stellar and planetary radii too high by a few percent.

With Kepler-47 b and c, six confirmed transiting circumbinary planets are currently known. Their orbital periods relative to their host binary stars show no tendency to be in resonance, and their radii are Saturn-size and smaller. Given that Jupiter-size planets are easier to detect, their absence in the Kepler data suggests that the formation and migration history of circumbinary planets may disfavor Jupiter-mass planets orbiting close to the stars, in accord with (22).

The planets in Kepler-47 are expected to have formed much farther out than their present orbits, at locations where the conditions for the formation of giant planets are more favorable (9, 10). The planets have likely migrated to their current orbits as a result of interactions with the circumbinary disk. The multiplicity and coplanarity of the orbits strengthen the argument for a single-disk formation and a migration scenario for circumbinary planetary systems. However, unlike

orbits around a single star, the environment around a binary star is much more dynamic and tends to augment planet-planet interactions. The relatively large distance between the orbits of the inner and outer planets in the Kepler-47 system is consistent with requirements for dynamical stability (23).

The previously detected transiting circumbinary planet systems show no evidence for more than a single planet. The multiplanet nature of the Kepler-47 system establishes that despite the chaotic environment around binary stars, planetary systems can form and persist close to the binary, and invites a broader investigation into how circumbinary planets compare to planets and planetary systems around single stars.

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## Supplementary Materials

[www.sciencemag.org/cgi/content/full/science.1228380/DC1](http://www.sciencemag.org/cgi/content/full/science.1228380/DC1)  
Materials and Methods  
Supplementary Text  
Figs. S1 to S25  
Tables S1 to S9  
References (25–59)

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# Quantum-Enhanced Optical-Phase Tracking

Hidehiro Yonezawa,<sup>1</sup> Daisuke Nakane,<sup>1</sup> Trevor A. Wheatley,<sup>1,2,3</sup> Kohjiro Iwasawa,<sup>1</sup> Shuntaro Takeda,<sup>1</sup> Hajime Arai,<sup>1</sup> Kentaro Ohki,<sup>4</sup> Koji Tsumura,<sup>5</sup> Dominic W. Berry,<sup>6,7</sup> Timothy C. Ralph,<sup>2,8</sup> Howard M. Wiseman,<sup>9\*</sup> Elanor H. Huntington,<sup>2,3</sup> Akira Furusawa<sup>1\*</sup>

Tracking a randomly varying optical phase is a key task in metrology, with applications in optical communication. The best precision for optical-phase tracking has until now been limited by the quantum vacuum fluctuations of coherent light. Here, we surpass this coherent-state limit by using a continuous-wave beam in a phase-squeezed quantum state. Unlike in previous squeezing-enhanced metrology, restricted to phases with very small variation, the best tracking precision (for a fixed light intensity) is achieved for a finite degree of squeezing because of Heisenberg’s uncertainty principle. By optimizing the squeezing, we track the phase with a mean square error  $15 \pm 4\%$  below the coherent-state limit.

There are many tasks where precise optical phase estimation is critical, including communication (1, 2) and metrology (3). Quantum mechanics imposes a fundamental bound on precision (4–6), and this already limits gravitational wave detection (7–9) and can guarantee security in quantum cryptography (10). The quantum limits are determined by optimizing (subject to

constraints) the input quantum state, the quantum measurement, and the data processing. Much effort has been devoted to approaching the fundamental quantum limits (3, 5, 6).

Phase estimation can be divided into two kinds (11): phase sensing, where the phase is known to always lie within some small interval [e.g., (12)], and general phase estimation, where

it is not so constrained [e.g., (11)]. In the former case, when (as in most practical situations) the field has a large coherent amplitude, the problem can be linearized in terms of the phase rotation (7), which greatly simplifies the task of optimizing the input state and the measurement. By contrast, in the case of unconstrained phase estimation the problem cannot be linearized, and as a consequence the optimization problem is considerably harder (11, 13–22). Although a quantum enhancement of phase sensing using nonclassical states of light has recently been demonstrated (8, 9), this has been done for general phase estimation only with postselected results (11).

We present a demonstration of unconstrained phase estimation with a quantum enhancement using nonclassical (squeezed) states. We used homodyne detection, with no postselection of data and no compensation for losses or detector inefficiency in the system. Moreover, the problem of a stochastically varying phase is addressed (8, 9, 19–22), as is highly relevant for physical metrology and communication, rather than a time-invariant (but initially unknown) phase (11–13, 15–18). To perform optimal estimation, we have implemented optical-phase tracking: a phase-lock loop that strives to maintain the maximum measurement sensitivity for a widely varying phase. The quantum noise in the photocurrent prevents the maximum sensitivity from being perfectly maintained, which is why we observe an optimal degree of squeezing.

Our experiment (Fig. 1A) used a continuous-wave optical phase-squeezed beam. The phase of the beam is modulated with the signal  $\varphi(t)$ , the waveform to be estimated (22–24). As in (21, 22), we used a stochastic waveform defined by

$$\varphi(t) = \sqrt{\kappa} \int_{-\infty}^t e^{-\lambda(t-s)} dV(s) \quad (1)$$

Here,  $dV(s)$  is a classically generated Wiener process (25) (white noise),  $\lambda^{-1}$  is the correlation time of  $\varphi(t)$ , and  $\kappa$  determines the magnitude of

the phase variation, which is of order unity. This  $\varphi(t)$  is a continuous-time random walk with a tendency to return to the mean phase of zero, a kind of noisy relaxation process that occurs in many physical situations (25).

The phase-modulated beam was measured by homodyne detection, using a local oscillator (LO) and yielding a noisy current  $I(t)$ . The LO phase  $\Phi(t)$  was feedback-controlled to be  $\Phi(t) \approx \pi/2 + \varphi(t)$ , because this is the most sensitive operating point for sensing changes in  $\varphi(t)$  (Fig. 1B). Because  $\varphi(t)$  is unknown, the best strategy is adaptive metrology (11, 13, 15–21), in which feedback control is used to set  $\Phi(t) = \varphi_f(t) + \pi/2$ , where  $\varphi_f(t)$  is a filtered estimate of  $\varphi(t)$ , that is, an estimate based on  $I(s)$  for all  $s < t$ . This gives a normalized homodyne output current  $I(t)$  of (19, 26),

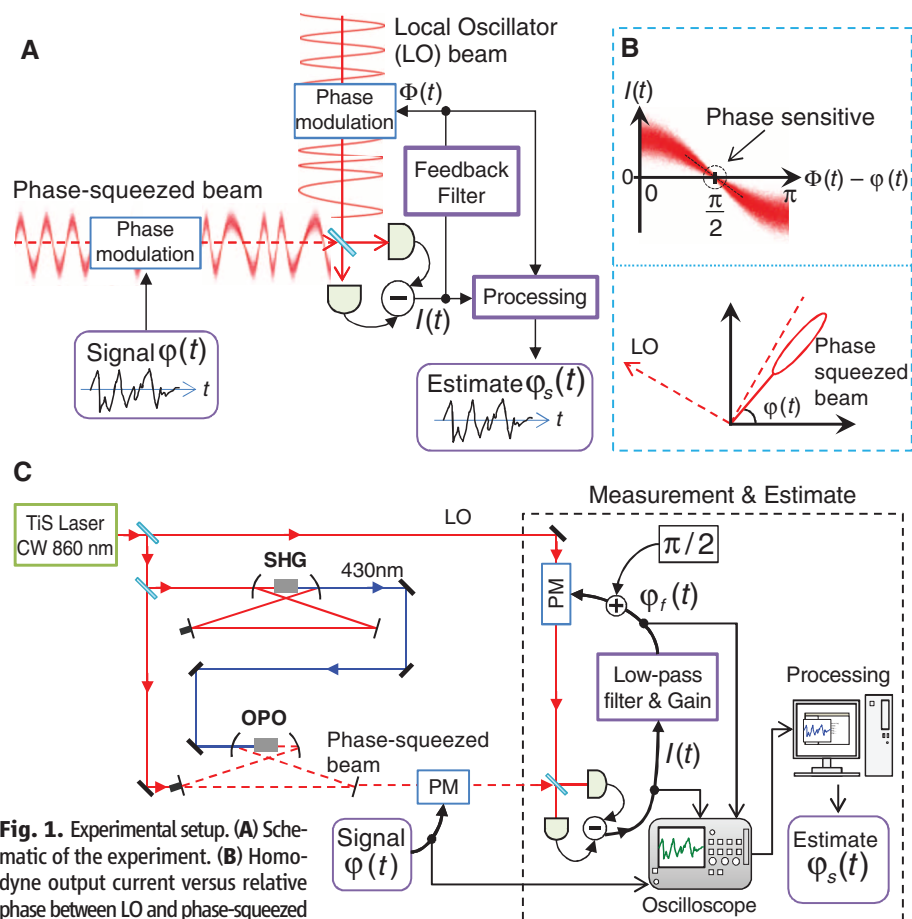
$$I(t)dt \simeq 2|\alpha|[\varphi(t) - \varphi_f(t)]dt + \sqrt{\bar{R}_{sq}}dW(t) \\ \bar{R}_{sq} = \sigma_f^2 e^{2r_p} + (1 - \sigma_f^2)e^{-2r_m} \quad (2)$$

Here,  $|\alpha|$  is the amplitude of the input phase-squeezed beam, and  $dW(t)$  is another Wiener process (25) arising from the squeezed vacuum fluctuations. The magnitude  $\bar{R}_{sq}$  of these quan-

tum fluctuations is determined by the degree of squeezing ( $r_m \geq 0$ ) and antisqueezing ( $r_p \geq r_m$ ) and by  $\sigma_f^2 = \langle [\varphi(t) - \varphi_f(t)]^2 \rangle$ . Note that several approximations, justified in (26), have been made to derive Eq. 2, most notably a second-order expansion for  $I(t)dt$  in the small variable  $[\varphi(t) - \varphi_f(t)]$ .

For optimal feedback control, the Kalman filter was used for  $\varphi_f(t)$  (22), which is the causal (i.e., real time) estimator with the lowest mean square error (MSE). The Kalman filter is the optimal filter for estimating  $\varphi(t)$  of the form of Eq. 1 when using a coherent beam (22), and the calculation generalizes to our squeezed case (26). Although the filtered estimate  $\varphi_f(t)$  is a good estimate of the signal phase  $\varphi(t)$ , to obtain the best estimate we applied the acausal technique of smoothing (21, 22, 24). After storing data over a certain period of time, a precise estimate of  $\varphi_s(t)$  was obtained at a time  $t$  in the middle of that period by using observations both before and after  $t$ . The MSE of the smoothed estimate  $\sigma_s^2 = \langle [\varphi(t) - \varphi_s(t)]^2 \rangle$  is given as (22, 26),

$$\sigma_s^2 = \kappa / \left( 2\sqrt{4\kappa|\alpha|^2 / \bar{R}_{sq} + \lambda^2} \right) \quad (3)$$



**Fig. 1.** Experimental setup. (A) Schematic of the experiment. (B) Homodyne output current versus relative phase between LO and phase-squeezed beam and phasor diagram for a slightly nonoptimal relative phase (as will occur in the phase-tracking problem). (C) Detail of the experimental setup. The abbreviations are TiS, titanium sapphire; CW, continuous-wave; PM, phase modulator; and SHG, second-harmonic-generation.

<sup>1</sup>Department of Applied Physics, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan. <sup>2</sup>Centre for Quantum Computation and Communication Technology, Australian Research Council, Canberra, Australia.

<sup>3</sup>School of Engineering and Information Technology, University College, The University of New South Wales, Canberra, ACT 2600, Australia. <sup>4</sup>Department of Applied Mathematics and Physics, Graduate School of Informatics, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan.

<sup>5</sup>Department of Information Physics and Computing, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. <sup>6</sup>Institute for Quantum Computing, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada. <sup>7</sup>Department of Physics and Astronomy, Macquarie University, NSW 2109, Australia.

<sup>8</sup>School of Mathematics and Physics, University of Queensland, Brisbane, QLD 4072, Australia. <sup>9</sup>Centre for Quantum Dynamics and Centre for Quantum Computation and Communication Technology, Griffith University, Brisbane, QLD 4111, Australia.

\*To whom correspondence should be addressed. E-mail: h.wiseman@griffith.edu.au (H.W.); akiraf@ap.t.u-tokyo.ac.jp (A.F.)

Recall that  $\bar{R}_{\text{sq}}$  (Eq. 2) is a function of  $\sigma_s^2$ , so the above expression for  $\sigma_s^2$  is still implicit. The full solutions are given in (26), but in the parameter regime of our experiment,  $\sigma_s^2$  is roughly proportional to  $\sqrt{\bar{R}_{\text{sq}}}$ . That is, by using a nonclassical beam with effective squeezing  $\bar{R}_{\text{sq}} < 1$  we expect to be able to overcome the coherent-state limit (CSL) by a factor of  $\sqrt{\bar{R}_{\text{sq}}}$ .

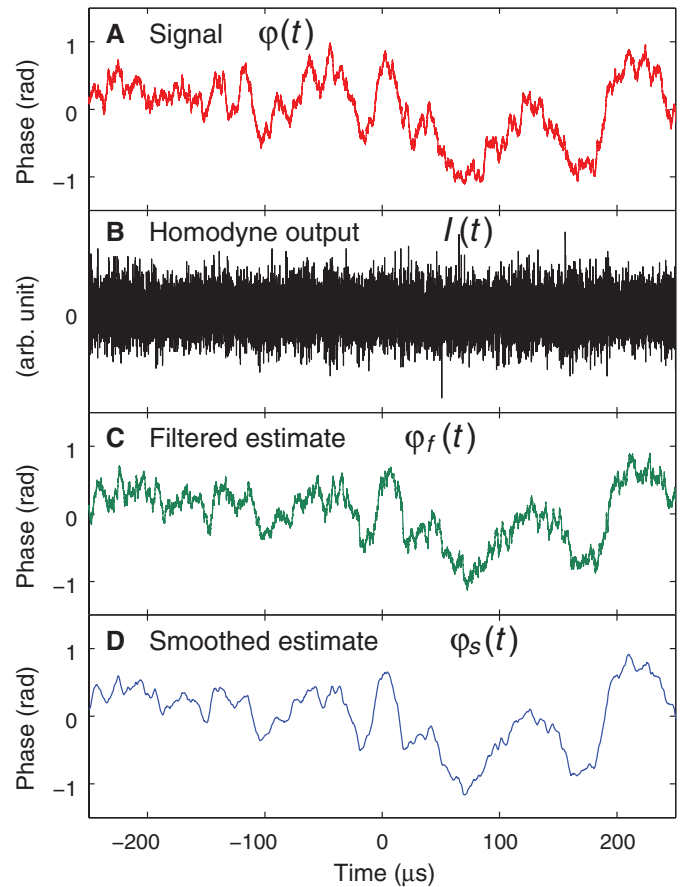
Our experiment (Fig. 1C) used an 860-nm continuous-wave titanium sapphire laser. The phase-squeezed beam was added by an optical parametric oscillator (OPO). The OPO was driven below threshold by a 430-nm pump beam, generated by a second-harmonic-generation cavity. We obtained up to  $-4$  dB of phase squeezing. The signal  $\varphi(t)$  was produced by a digital random signal generator and a low-pass filter with a cutoff frequency of  $\lambda/2\pi$ . This was imposed on the phase-squeezed beam by using an electrooptic modulator. Homodyne detection was performed on this phase-modulated beam with an overall efficiency of  $\eta = 0.85$ . The homodyne current went to the optimal feedback filter [another low-pass filter with a cutoff frequency  $\lambda/2\pi$  (26)]. Its output,  $\varphi_f(t)$ , was then shifted by  $\pi/2$  and applied on the phase of the LO beam with another electrooptic modulator.

We recorded  $\varphi(t)$ ,  $I(t)$ , and  $\varphi_f(t)$  by an oscilloscope with a sampling rate of 100 MHz. Figure 2 shows a typical segment of the recorded signals, plus the smoothed estimate  $\varphi_s(t)$ . The parameters here are  $\kappa = 1.9 \times 10^4 \pm 0.1 \times 10^4$  rad/s,  $\lambda = 5.9 \times 10^4 \pm 0.5 \times 10^4$  rad/s,  $|\alpha|^2 = 1.00 \times 10^6 \pm 0.06 \times 10^6$  s $^{-1}$ , squeezing  $-3.1 \pm 0.1$  dB ( $r_m = 0.36 \pm 0.01$ ) and antisqueezing  $5.1 \pm 0.1$  dB ( $r_p = 0.59 \pm 0.01$ ), from a pump beam power of 80 mW. The uncertainties represent 1 standard deviation.  $\kappa$  and  $\lambda$  are fixed through this paper. The current  $I(t)$  has zero mean because the feedback loop is designed to operate the homodyne measurement at this point of maximum sensitivity (Fig. 1B). Although the filtered estimate  $\varphi_f(t)$  has a visible delay because of its causal nature, the smoothed estimate  $\varphi_s(t)$  does not, and the signal phase  $\varphi(t)$  is reliably tracked.

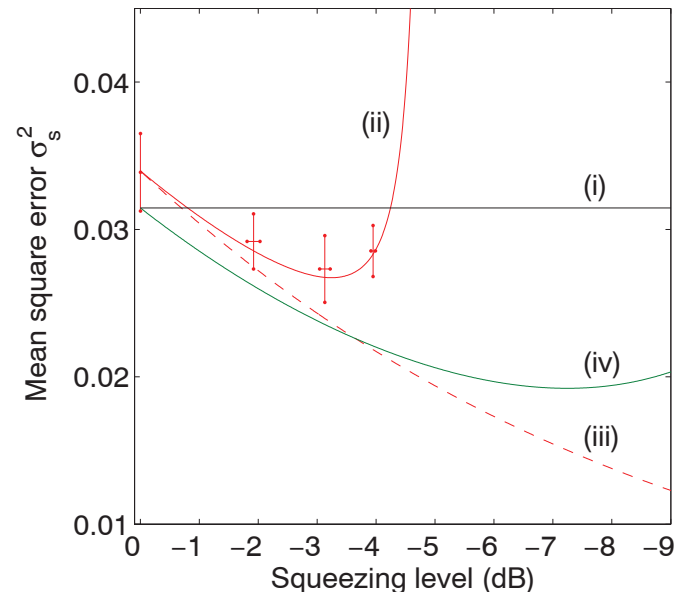
To investigate the squeezing enhancement, we performed phase tracking with a fixed square amplitude  $|\alpha|^2 = 1.00 \times 10^6 \pm 0.06 \times 10^6$  s $^{-1}$  but with varying squeezing levels arising from OPO pump beam powers of 0, 30, 80, and 180 mW. Independently of the phase estimation, squeezing and antisqueezing levels were measured for each pump beam power (26). The red crosses in Fig. 3 show the MSEs of the smoothed estimates  $\sigma_s^2$  as a function of the squeezing level. The MSE was calculated from 2 ms of data ( $2 \times 10^5$  samples). Repeating this 15 times gave the average MSE and its standard deviation.

Figure 3 shows three key results. First, the squeezing enhancement is verified: The MSEs are reduced below the CSL (i) by using phase-squeezed beams. Second, the experimental results are in good agreement with the prediction (ii) and in disagreement with the theory curve (iii), which is based on approximating the homo-

**Fig. 2.** Time domain results of phase estimate. (A) Signal phase to be estimated  $\varphi(t)$ . (B) Homodyne output current  $I(t)$ . (C) Filtered estimate  $\varphi_f(t)$ . (D) Smoothed estimate  $\varphi_s(t)$ .



**Fig. 3.** Smoothed MSE  $\sigma_s^2$  versus squeezing level. Red crosses represent experimental data. Trace (i) is the coherent-state limit, which is reachable with a coherent beam only if we have unit-detection efficiency  $\eta = 1$ . Trace (ii) is the theoretical curve from Eq. 3. Trace (iii) is the theoretical curve based on approximating the homodyne output current  $I(t)$  to only first order in  $[\varphi(t) - \varphi_f(t)]$  so that  $\bar{R}_{\text{sq}} = e^{-2r_m}$ . Trace (iv) is the theoretical curve from Eq. 3 for pure squeezed beams (i.e., without loss).

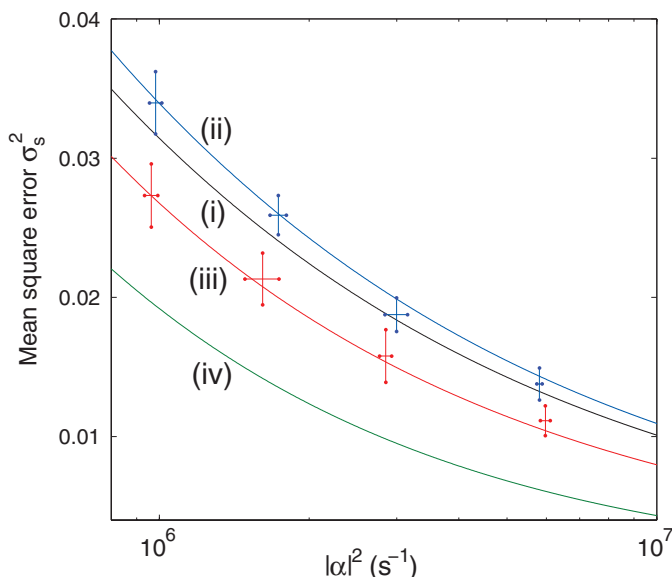


dyne output current  $I(t)$  to only first order in  $[\varphi(t) - \varphi_f(t)]$  so that  $\bar{R}_{\text{sq}} = e^{2r_m}$ . Third, at the higher squeezing level the MSE is saturated, indicating the existence of an optimal squeezing level. Even in the theoretical curve (iv), for pure squeezed beams and zero loss, the MSE has a minimum. This curve corresponds to the fundamental limit imposed by Heisenberg's uncertainty

principle for the phase and amplitude quadratures, namely  $e^{-2r_p} \times e^{2r_m} \geq 1$ . Although more squeezing decreases the  $e^{-2r_m}$  term in  $\bar{R}_{\text{sq}}$ , it increases the  $e^{2r_p}$  term because the tracking is imperfect, which itself is a consequence of the noise in the photocurrent (Eq. 2). This defines (self-consistently) the optimal degree of squeezing, which depends on the parameters  $|\alpha|$ ,  $\kappa$ , and  $\lambda$  (26).



**Fig. 4.** Dependence of the smoothed MSE  $\sigma_s^2$  on the amplitude squared  $|\alpha|^2$ . Blue and red crosses are experimental data for coherent and squeezed beams, respectively. Trace (i) is the coherent-state limit. Trace (ii) is the theoretical curve for coherent beams with the experimental setup (i.e., including inefficiency). Trace (iii) is the theoretical curve for squeezed beams, including inefficiency. Trace (iv) is the theoretical curve for pure squeezed beams and 100% efficiency, with the squeezing level optimized for each  $|\alpha|^2$ .



Experimentally, we varied the amplitude  $|\alpha|$  while fixing the pump beam power to 80 mW, giving squeezing and antisqueezing levels of  $-3.2 \pm 0.2$  dB and  $4.9 \pm 0.3$  dB, respectively. Theoretically, the optimal squeezing level increases with  $|\alpha|$ , and so too does the squeezing enhancement, without limit. However, for our experimental conditions ( $10^6 \text{ s}^{-1} \leq |\alpha|^2 < 10^7 \text{ s}^{-1}$ ) the effect of keeping the squeezing fixed is minor (less than 3% difference to  $\sigma_s^2$ ). Figure 4 shows the dependence of the MSE  $\sigma_s^2$  on  $|\alpha|$ . The theoretical curves show good agreement with experiments. Over the whole amplitude range, the estimates with the squeezed beams surpass what is possible with coherent states, with  $\sigma_s^2$ , averaged over the four different amplitudes, being  $15 \pm 4\%$  below the CSL. The conclusion is essentially unaltered if one calculates the CSL not in terms of  $|\alpha|^2$  but in terms of the effective photon flux  $\mathcal{N}_{\text{eff}}$ , which equals  $|\alpha|^2$  plus the extra photons resulting from the squeezed vacuum fluctuations in the relevant spectral range (26).

We have tracked the phase of a squeezed optical field that varies stochastically in time over a substantial angular range. Our use of Kalman filtering in real-time adaptive measurements of nonclassical systems could be applied also in solid-state and nanomechanical devices. Optimizing both the degree of squeezing and its bandwidth according to the experimental conditions would allow a completely rigorous treatment of photon flux. Lower losses and more squeezing would then enable a dramatic improvement to a precision that scales differently with photon flux, with  $\sigma^2 \propto \mathcal{N}^{-5/8}$  (20) as opposed to the  $\sigma^2 \propto \mathcal{N}^{-1/2}$  in the current setup.

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#### Supplementary Materials

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## Subtractive Patterning via Chemical Lift-Off Lithography

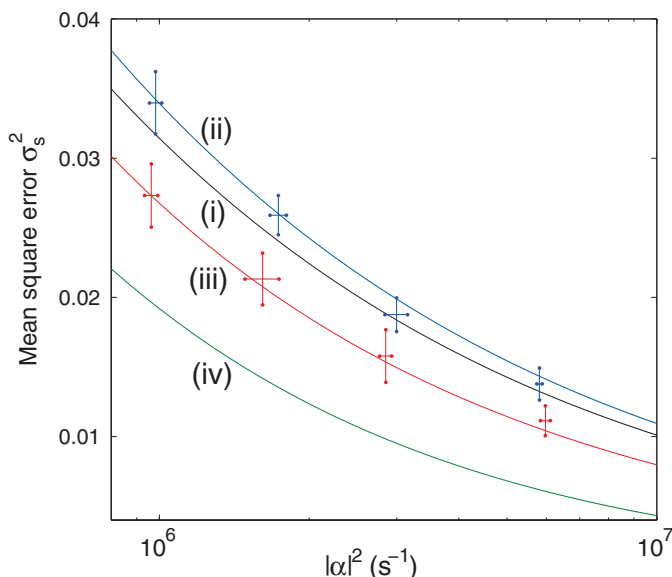
Wei-Ssu Liao,<sup>1,2</sup> Sarawut Cheunkar,<sup>1,3</sup> Huan H. Cao,<sup>1,2</sup> Heidi R. Bednar,<sup>1,2</sup> Paul S. Weiss,<sup>1,2,3,4,5\*</sup> Anne M. Andrews<sup>1,2,6,7\*</sup>

Conventional soft-lithography methods involving the transfer of molecular “inks” from polymeric stamps to substrates often encounter micrometer-scale resolution limits due to diffusion of the transferred molecules during printing. We report a “subtractive” stamping process in which silicone rubber stamps, activated by oxygen plasma, selectively remove hydroxyl-terminated alkanethiols from self-assembled monolayers (SAMs) on gold surfaces with high pattern fidelity. The covalent interactions formed at the stamp-substrate interface are sufficiently strong to remove not only alkanethiol molecules but also gold atoms from the substrate. A variety of high-resolution patterned features were fabricated, and stamps were cleaned and reused many times without feature deterioration. The remaining SAM acted as a resist for etching exposed gold features. Monolayer backfilling into the lift-off areas enabled patterned protein capture, and 40-nanometer chemical patterns were achieved.

High-throughput molecular printing strategies with high feature resolution are central goals for lithography. However, progress has been impeded by the conflicting aims of large-area fabrication versus precision, and of convenience versus cost (1–4). For instance,

although photolithography enables patterning over large areas (centimeters), the prototyping process is time-consuming and resolution is restricted by light diffraction (1–3). Patterning by electron beam lithography (EBL) or scanning probe lithography (SPL) techniques, such as dip-pen nanolithography,

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## Subtractive Patterning via Chemical Lift-Off Lithography

Wei-Ssu Liao,<sup>1,2</sup> Sarawut Cheunkar,<sup>1,3</sup> Huan H. Cao,<sup>1,2</sup> Heidi R. Bednar,<sup>1,2</sup> Paul S. Weiss,<sup>1,2,3,4,5\*</sup> Anne M. Andrews<sup>1,2,6,7\*</sup>

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High-throughput molecular printing strategies with high feature resolution are central goals for lithography. However, progress has been impeded by the conflicting aims of large-area fabrication versus precision, and of convenience versus cost (1–4). For instance,

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nanoshaving, and nanografting (5–7), produces high-resolution features (<10 nm and <100 nm for EBL and SPL, respectively) (1–3), but throughput is limited by serial processing speeds.

Soft-lithography strategies produce patterns over large areas at the micro- and nanoscales (1, 3, 4, 8–10). Commercial polymers (such as polydimethylsiloxane, PDMS) are used as molds for pattern transfer via contact printing. The bas-relief pattern on a master mold is fabricated by photolithography for large-area patterning or EBL for high-resolution patterning (1, 3). Once the master is generated, patterned features are negatively transferred to PDMS stamps, which are then “inked” with organic molecules, proteins, nanoparticles, or DNA (1, 10–16).

Among the materials transferred, organic molecules such as alkanethiols and other related molecules, which form self-assembled monolayers (SAMs) on Au substrates, can be readily subjected to chemical modification at the exposed terminal groups for capturing biomolecules (1, 16–18). Moreover, SAMs serve as “molecular resists” against different wet etchants, enabling patterns to be transferred reproducibly to underlying substrates (19). However, the success of contact printing and related soft-lithography techniques is also limited by the chemistries and compatibility of the inks, stamps, and substrates (1, 3, 4). For example, lateral diffusion and gas-phase deposition of ink molecules tend to reduce pattern fidelity (20, 21), creating a resolution limit of ~100 nm for alkanethiols on Au.

To overcome the limitations of stamp feature replication in soft lithography, the general principles of contact printing must be modified to achieve sharp, stable, and reproducible chemical features on substrates (7, 19, 22, 23). We transformed the conventional contact printing process such that the polymer stamp is activated and then used to lift off a preformed SAM resist. A strong contact-induced interaction at the stamp-SAM interface enables the transfer of sharp stamp features by mechanical desorption of resist only in the areas of stamp-substrate contact. The subtractive nature of this process precisely replicates features from the master mold (9, 24). This approach, chemical lift-off lithography (CLL), facilitates the addition of different molecules into the lift-off areas to produce multicomponent patterned SAMs. It also enables the intact areas to

act as an etch resist for the transfer of features to the underlying substrate. Moreover, stamps used for CLL can be cleaned and reused many times without deterioration.

Alkanethiols with different terminal groups (Table 1) were used to form SAMs on Au-coated Si substrates. Soft-lithography stamps were created from PDMS to transfer features of different geometries from master molds (fabricated by standard photolithography and EBL techniques) to the molecular-resist layers (1, 8, 10). The CLL process is outlined schematically in Fig. 1. A PDMS stamp was first activated by exposure to oxygen plasma, yielding a fully hydrophilic and reactive surface (17, 25–27). The stamp and SAM-modified substrate were then brought into conformal contact. The stamp was peeled away from the substrate, which removed resist molecules selectively in the areas contacted by the stamp, transferring stamp features with high resolution to the substrate.

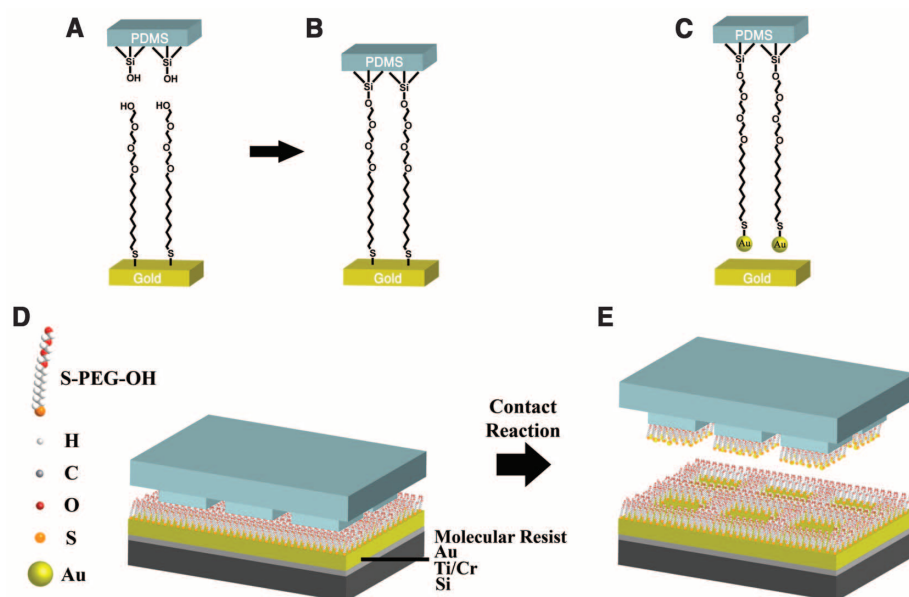
On the basis of earlier work, we hypothesized that the Au-Au bonds in the substrate metal lattice, rather than the Au-S bonds between the substrate and alkanethiol, are preferentially broken

during lift-off. The breaking of Au-Au bonds during SAM desorption has been a particular subject of controversy (6, 28–32). The mobility of Au thiols within SAMs (29, 33, 34) indicates that weak Au-Au bonds are present at the substrate surface. Furthermore, recent studies show the presence of Au adatoms beneath SAMs, which leads to facile Au-Au bond breakage because of reduced coordination of the adatoms (35–38). We made a featureless, oxygen plasma-treated PDMS stamp and brought it into contact with a hydroxyl-terminated SAM-coated Au surface. After lift-off, a peak indicating the presence of Au was observed on the PDMS stamp surface by x-ray photoelectron spectroscopy (XPS; see spectra in fig. S1). This finding is consistent with Au being removed from the underlying substrate (39).

The presence of Au on oxygen plasma-treated PDMS surfaces after chemical lift-off led us to propose that a contact-induced chemical reaction between the hydrophilic stamp surface and the molecular-resist layer results in Au-Au bond rupture during stamp removal. Studies have shown that oxygen plasma treatment yields siloxyl

**Table 1.** Alkanethiol molecules and terminal groups used in chemical lift-off lithography.

Alkanethiol	Chemical formula
Hydroxyl-terminated tri(ethylene glycol)undecanethiol (TEG)	$\text{HS}-(\text{CH}_2)_{11}-(\text{C}_2\text{H}_4\text{O})_3-\text{OH}$
Biotin-terminated hexa(ethylene glycol)undecanethiol	$\text{HS}-(\text{CH}_2)_{11}-(\text{C}_2\text{H}_4\text{O})_6-\text{NH}-\text{C}_{10}\text{H}_{15}\text{O}_2\text{N}_2\text{S}$
Hydroxyl-terminated undecanethiol	$\text{HS}-(\text{CH}_2)_{11}-\text{OH}$
Methyl-terminated undecanethiol	$\text{HS}-(\text{CH}_2)_{11}-\text{CH}_3$
Methoxy-terminated tri(ethylene glycol)undecanethiol	$\text{HS}-(\text{CH}_2)_{11}-(\text{C}_2\text{H}_4\text{O})_3-\text{O}-\text{CH}_3$



**Fig. 1.** Schematic illustration of the molecular-resist lift-off process. (A) A polydimethylsiloxane (PDMS) stamp is activated by oxygen plasma treatment, producing hydrophilic siloxyl groups. (B) A surface-induced contact reaction is implemented via close contact between the stamp and hydroxyl-terminated molecules self-assembled on an Au substrate. (C) Stamp removal lifts off resist molecules and underlying Au. (D) In chemical lift-off lithography (CLL), a patterned PDMS stamp is brought into conformal contact with a self-assembled molecular resist. (E) Lift-off is limited to the stamp-contact regions.

<sup>1</sup>California NanoSystems Institute, University of California, Los Angeles, CA 90095, USA. <sup>2</sup>Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA. <sup>3</sup>Department of Chemistry, Pennsylvania State University, University Park, PA 16802, USA. <sup>4</sup>Department of Materials Science and Engineering, University of California, Los Angeles, CA 90095, USA. <sup>5</sup>Department of Physics, Pennsylvania State University, University Park, PA 16802, USA. <sup>6</sup>Department of Psychiatry and Biobehavioral Health, University of California, Los Angeles, CA 90095, USA. <sup>7</sup>Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, CA 90095, USA.

\*To whom correspondence should be addressed. E-mail: aandrews@mednet.ucla.edu (A.M.A.); psw@cnsi.ucla.edu (P.S.W.)



groups on PDMS stamp surfaces, which facilitate condensation reactions between Si-OH and hydroxyl groups on different oxides, such as Au, Ti, and Si to form Si-O-Au, Si-O-Ti, and Si-O-Si linkages, respectively (9, 24, 40–42). We anticipated that the same type of linkage (Si-O-SAM) would be established between Si-OH groups on oxygen plasma-treated PDMS stamp surfaces and hydroxyl-terminated groups on SAMs.

To investigate the roles of the molecular resist tail groups in the CLL process, we assembled two different hydroxyl-terminated alkanethiol mol-

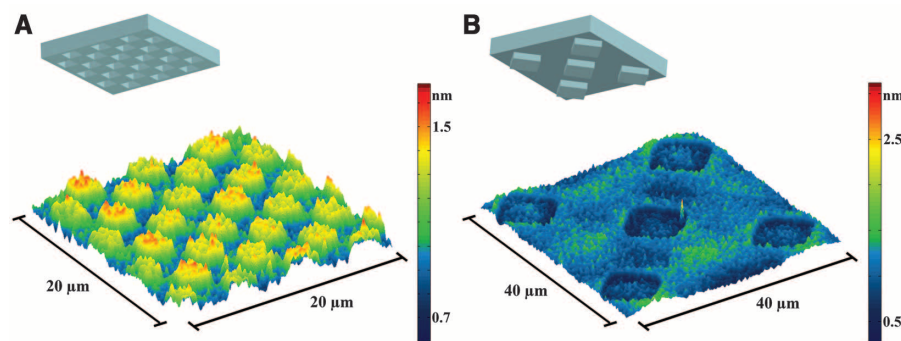
ecules, with and without oligo(ethylene glycol), as molecular resist monolayers (Table 1). Both provided good transfer of stamp features to SAM-coated Au substrates (Fig. 2 and fig. S5A). In contrast, when methoxy- or methyl-terminated alkanethiol molecules (Table 1) were tested under the same assembly and lift-off conditions, no detectable transfer of stamp features was found on SAM-coated Au surfaces (figs. S5B and S5C, respectively). Stamp features were not transferred when a hydrophilic PDMS stamp was used directly with a bare Au substrate (fig. S5D). Thus,

tail group reactivity dictates whether lift-off occurs via hydrophilic PDMS stamps.

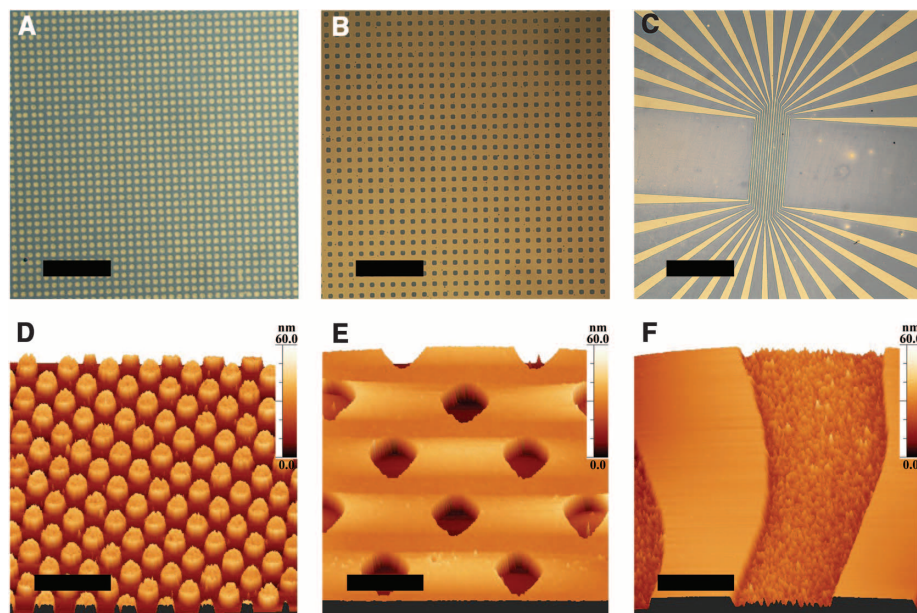
Fourier-transform infrared reflection absorption spectroscopy (FT-IRRAS) was used to investigate the extent of lift-off occurring in a prototypical SAM. Spectral analysis indicated that 75 to 80% of hydroxyl-terminated undecanethiol molecules are removed after the lift-off process (fig. S6). Previous reactive patterning of hydrogen-bonding SAMs showed that this level of damage makes the SAM labile to complete displacement, and the hydrogen bonding in the intact areas prevents diffusion and thus pattern dissolution (43). The terminal functionality of the initial SAM influences lift-off via the extent of the contact-induced reaction at the SAM-stamp interface. Lift-off from SAMs of hydroxyl-terminated tri(ethylene glycol)alkanethiol (TEG) was sufficient to enable patterning of underlying substrates by wet etching and to produce patterned multi-component SAMs capable of biorecognition (see below).

Chemical patterns of TEG were characterized by atomic force microscopy (AFM) and bright-field optical microscopy, as shown in Figs. 2 and 3. Stamps with depressed well-like motifs or protruding posts were used to create different surface relief patterns. The stamp negative was produced in the resist, as molecules were removed (instead of added) by patterning. For example, islands of SAM resist remained when a stamp with a depressed relief was used; the areas surrounding the relief on the stamp contacted the SAM surface, and the molecular resist was removed in these areas during the lift-off step. The AFM topographic image in Fig. 2A illustrates the protruding SAM islands after patterning. By contrast, well-shaped features were observed on the substrate when a stamp with a protruding relief was used for patterning (Fig. 2B). In Fig. 2, AFM topography profiles indicate  $2.0 \pm 0.3$  nm differences between lift-off and non-lift-off areas. The thickness of the TEG SAMs was  $1.6 \pm 0.1$  nm by ellipsometry. The difference can be accounted for by a single atomic layer of Au removed during the lift-off process.

We explored the use of the intact SAM areas as an unconventional resist to transfer patterns to the underlying material, Au, through selective wet chemical etching (19, 44). Exposed areas of the Au surface were contacted by the etchant solution while the intact SAM molecular resist protected the remaining regions of Au. Etchant solutions removed exposed Au via oxidation by  $\text{Fe}^{3+}$ , followed by complexation and dissolution of oxidized metal by thiourea (45). A variety of patterns (inverse replicas of the PDMS stamp features) with features of different sizes were transferred, including lines, holes, and pillars (Fig. 3). The advantages of large patterning areas and high-fidelity features are apparent in the bright-field images (Fig. 3, A to C) and AFM topography images (Fig. 3, D to F), respectively. Differences in AFM heights indicate that features have been transferred to the level of the underlying



**Fig. 2.** (A and B) Atomic force microscope topographic images of substrates patterned by CLL. Self-assembled monolayers of hydroxyl-terminated tri(ethylene glycol)alkanethiol on Au substrates were patterned using CLL and a PDMS stamp with depressed wells ( $2 \mu\text{m}$  by  $2 \mu\text{m}$ ) (A) or a PDMS stamp with protruding posts ( $10 \mu\text{m}$  by  $10 \mu\text{m}$ ) (B). Stamp geometries are illustrated above the images. Contact dwell time was 5 min. AFM topographical heights are shown in the scale bars to the right of each image.



**Fig. 3.** Patterning underlying gold substrates by CLL. Hydroxyl-terminated tri(ethylene glycol)undecanethiol was self-assembled on Au substrates. Lift-off lithography via activated PDMS stamps was used to produce a variety of patterns. Substrates were then chemically etched ( $\text{Fe}^{3+}$ /thiourea) to pattern the underlying metal by removing additional gold in the exposed regions. The SAM molecular resist was intact during imaging with bright-field microscopy and AFM. Patterns transferred by the molecular-resist lift-off process include pillars (A and D), wells (B and E), and channels (C and F). Bright-field microscope images are shown in (A) to (C); corresponding AFM topography images are shown in (D) to (F). Scale bars,  $18 \mu\text{m}$  (A),  $130 \mu\text{m}$  (B),  $1325 \mu\text{m}$  (C),  $5 \mu\text{m}$  (D),  $15 \mu\text{m}$  (E), and  $17.5 \mu\text{m}$  (F). AFM topographical heights are shown in the upper right corners of (D) to (F).

substrate at a depth of 30 nm—the thickness of the original Au layer.

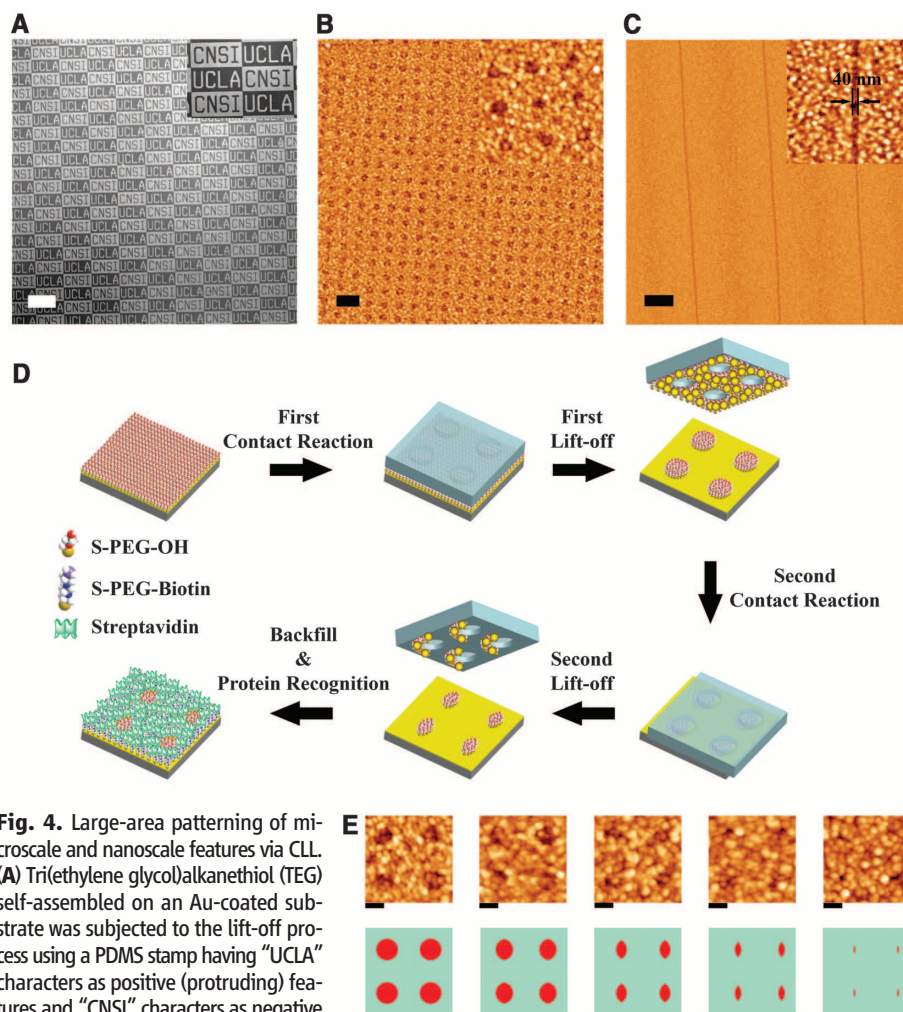
In addition to transferring patterns to SAMs and underlying Au substrates, CLL enables a SAM of a different composition to be assembled on the lift-off areas. Figure 4A shows a large-area, high-fidelity pattern of streptavidin binding to a biotinylated pattern created by lifting off areas of an initial TEG SAM to expose fresh Au substrate underneath. The substrate was then exposed to 90:10 TEG/biotin-terminated hexa(ethylene glycol)alkanethiol (Table 1) to produce a low-density biotinylated patterned SAM (17, 18). Streptavidin was captured from solution by surface-tethered biotin. Bound streptavidin was detected by fluorescence microscopy of fluorescein isothiocyanate (FITC)-conjugated antibodies against streptavidin. The bright fluorescent regions in Fig. 4A and its inset display the lift-off areas where biotin-terminated alkanethiols were backfilled and used to capture streptavidin from solution. The dark regions display minimal fluorescence because of the absence of biotin-terminated alkanethiol and the resistance to nonspecific protein adsorption by TEG (17, 46). The fabrication of biotin-streptavidin patterns demonstrates not only that CLL transfers large-area, high-fidelity patterns to SAMs, but also that the Au areas exposed after lift-off are advantageous for producing multiplexed bioselective patterned surfaces.

To carry out nanometer-scale chemical patterning, we implemented the lift-off process for biotin-streptavidin described above, using a PDMS stamp with 90-nm well-like features (Fig. 4B). Areas surrounding the wells were lifted off and backfilled with biotin-terminated alkanethiol to capture streptavidin, whereas the areas inside the wells were not removed, producing TEG islands. In one method to achieve features smaller than 90 nm, a double lift-off strategy was used in which the PDMS stamp was twice brought into conformal contact with the substrate (Fig. 4E). The initial lift-off step removed the molecules in the areas surrounding the stamp wells, leaving the TEG SAM inside the wells intact. During the second lift-off step, the stamp was offset with respect to the first pattern. (This result was initially a serendipitous consequence of being unable to maintain exact registry between multiple stamping steps.) Additional areas of the TEG SAM were removed, depending on the amount of registration. The exposed Au surfaces resulting from both TEG removal steps were backfilled with biotin-terminated alkanethiol. Figure 4E illustrates decreasing registration associated with smaller feature sizes. The resulting intact TEG regions formed increasingly narrow marquis-shaped features with decreased spacing between biotin-streptavidin molecular recognition areas. Note that if conventional contact printing were used in this case, lateral diffusion of ink molecules would blur nanospaced features beyond detection by AFM (47). In Fig. 4C, sharp features  $40 \pm 2$  nm in width were directly fabricated using a stamp with 40-nm channels, indicating that we have not

yet reached the resolution limit of the CLL method. Exploring the effects of Au grain size will also be important for future mechanistic studies and possible further improvement of nanoscale feature resolution.

Lateral diffusion of ink molecules, which occurs during increasing stamp contact times and/or molecular ink concentrations for additive printing methods on bare Au substrates, is avoided in CLL.

Preformed well-ordered SAMs, strong intermolecular interactions between hydrophilic SAM molecules, and a diffusion barrier created by the Au step edges (48) formed during lift-off prevent pattern dissolution. Patterned TEG SAMs produced by CLL showed no discernable dissolution after 2 days under ambient storage conditions (fig. S8). Furthermore, the backfilled multicomponent SAMs shown in Fig. 4 were produced by solution



**Fig. 4.** Large-area patterning of microscale and nanoscale features via CLL. (A) Tri(ethylene glycol)alkanethiol (TEG) self-assembled on an Au-coated substrate was subjected to the lift-off process using a PDMS stamp having “UCLA” characters as positive (protruding) features and “CNSI” characters as negative (depressed) features. After patterning,

a new monolayer of 90% TEG/10% biotin-terminated oligo(ethylene glycol)alkanethiol (nominal solution ratio) was self-assembled on the exposed Au regions (“UCLA” characters and areas surrounding the “CNSI” characters). Bright areas indicate fluorescence associated with FITC-labeled anti-streptavidin antibody recognition of streptavidin bound to biotin. Dark areas display minimal fluorescence due to the protein-resistant characteristics of TEG. The fluorescent pattern is sharp and extends over a large substrate area ( $>3$  mm<sup>2</sup>). Scale bar (main image), 250  $\mu$ m. (B) Au-coated substrates coated with TEG self-assembled monolayers were subjected to the lift-off process using a PDMS stamp with holes 90 nm in diameter. After patterning, a new monolayer of 100% biotin-terminated oligo(ethylene glycol)alkanethiol was self-assembled on the exposed Au regions (areas surrounding the resulting pillar features). Scale bar (main image), 400 nm. The inset shows a high-resolution AFM image of biospecific 90-nm circular features produced by CLL. (C) AFM images display biotin-streptavidin recognition areas separated by narrow line features. The inset shows a detailed AFM image of an individual line feature (width  $40 \pm 2$  nm) made using a stamp with 40-nm channels. Scale bar (main image), 1  $\mu$ m. (D) A PDMS stamp with holes 90 nm in diameter was brought into conformal contact once with a TEG SAM (upper left). In this case, substrates were stamped twice with decreasing registry (subsequent images from left to right). Patterned substrates were backfilled with biotin-terminated alkanethiol. (E) Topographic AFM images display decreasing feature sizes (from left to right):  $90 \pm 5$  nm,  $80 \pm 3$  nm,  $50 \pm 2$  nm,  $30 \pm 3$  nm, and  $15 \pm 5$  nm. Protruding (lighter) areas indicate biotin-streptavidin recognition. Shallow (darker) areas comprise intact TEG SAM. Scale bars, 100 nm.



deposition of the second SAM component over 12 hours; sharp pattern features were produced even in this case, arguing against diffusion or dissolution of the original lift-off pattern.

We investigated the time needed for the contact-induced chemical reaction at the stamp-substrate interface by examining 1-min versus 5-min contact times between oxygen plasma-treated PDMS stamps and hydroxyl-terminated, alkanethiol-coated Au surfaces. Features were transferred even with 1-min contact times; however, shorter contact times resulted in poor features produced after wet etching. Additionally, pattern transfer was maintained with short SAM deposition times. Hydroxyl-terminated alkanethiol SAMs formed during 1 hour of deposition were found to provide good transfer of stamp features to Au substrates, comparable to transfer obtained from SAMs formed overnight. These findings demonstrate advantages associated with short contact and SAM formation times for facilitating robust, expeditious, and high-throughput patterning by CLL. Ultimately, limits for SAM deposition and stamp contacts times will depend on the specific molecules used for SAM formation.

With this method, conventional nanolithographic patterning techniques such as photolithography and electron-beam lithography need only be used for the fabrication of stamp master molds. Once individual masters are produced, CLL can be implemented as a strategy for high-resolution, high-throughput, low-cost pattern fabrication. Because CLL enables patterns to be transferred to underlying substrates and can be used in a multiple-stamping strategy to produce patterns that are smaller than the actual stamp features, possible applications of CLL include the production of high-fidelity nanometer-scale patterns on Au substrates, as well as patterning of different materials such as Si, Ge, Pd, Pt, and graphene.

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## Supplementary Materials

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Materials and Methods  
Supplementary Text  
Figs. S1 to S8  
References (49, 50)

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# Pulsating Tubules from Noncovalent Macrocycles

Zhegang Huang,<sup>1,3</sup> Seong-Kyun Kang,<sup>1</sup> Motonori Banno,<sup>2</sup> Tomoko Yamaguchi,<sup>2</sup> Dongseon Lee,<sup>1</sup> Chaok Seok,<sup>1</sup> Eiji Yashima,<sup>2</sup> Myongssoo Lee<sup>1\*</sup>

Despite recent advances in synthetic nanometer-scale tubular assembly, conferral of dynamic response characteristics to the tubules remains a challenge. Here, we report on supramolecular nanotubules that undergo a reversible contraction-expansion motion accompanied by an inversion of helical chirality. Bent-shaped aromatic amphiphiles self-assemble into hexameric macrocycles in aqueous solution, forming chiral tubules by spontaneous one-dimensional stacking with a mutual rotation in the same direction. The adjacent aromatic segments within the hexameric macrocycles reversibly slide along one another in response to external triggers, resulting in pulsating motions of the tubules accompanied by a chiral inversion. The aromatic interior of the self-assembled tubules encapsulates hydrophobic guests such as carbon-60 (C<sub>60</sub>). Using a thermal trigger, we could regulate the C<sub>60</sub>-C<sub>60</sub> interactions through the pulsating motion of the tubules.

**S**elf-assembly of small molecular modules into tubules with hollow cavities is a key structural feature of living systems, as exemplified by tobacco mosaic virus and cytoplas-

mic microtubules (1, 2). Inspired by the biological systems, numerous efforts have been devoted to the design of synthetic building blocks that can form such hollow nanostructures through orchestrated interplay of various noncovalent interactions (3). Synthetic tubules have previously been fashioned by self-assembly of lipid molecules (4), aromatic amphiphiles (5–9), and oligopeptides (10–12). The stacking of ring-shaped compounds is an alternative way to construct tubular structures (13, 14). An example is provided by doughnut-like toroidal proteins that stack on top of one

<sup>1</sup>Department of Chemistry, Seoul National University, Seoul 151-747, Korea. <sup>2</sup>Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan. <sup>3</sup>Department of Chemistry, Harbin Institute of Technology, Harbin, 150001, P. R. China.

\*To whom correspondence should be addressed. E-mail: myongssoo@snu.ac.kr



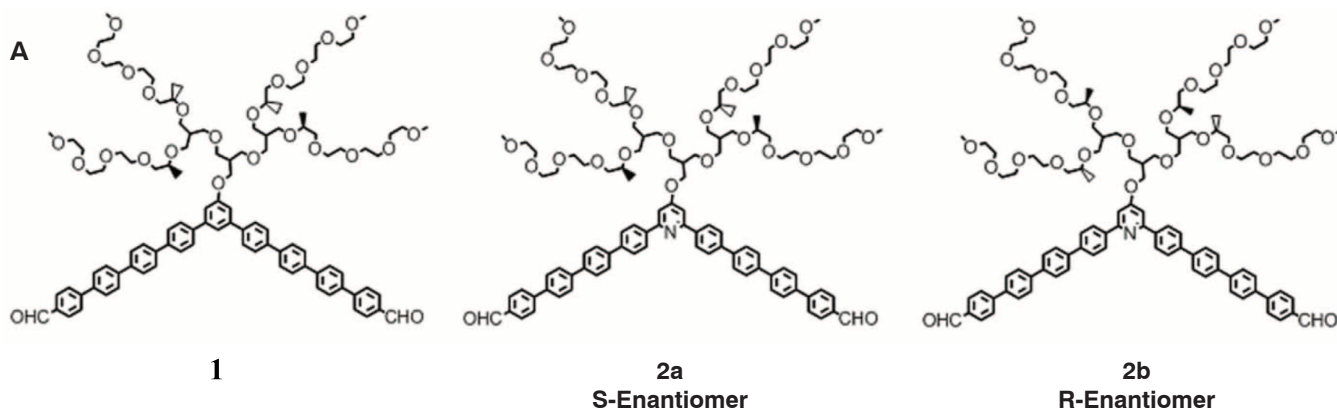
another, triggered by disulfide bond formation to create stable nanotubes (15, 16). Nonetheless, integration of dynamic response characteristics into synthetic tubules is limited. Stimuli-responsive action is known to occur in protein rings in vivo, but their one-dimensional (1D) fixation into tubules normally requires chemical reactions incompatible with dynamic motion (15–17). Lipid tubules face similar challenges because external triggers lead to the collapse of their internal cavities due to poor aggregation stability (4). The tubules formed by stacking of covalent macrocycles normally require hydrogen bonds too inflexible to undergo dynamic structural changes without breaking (11, 13).

The approach to tubule design presented here overcomes these limitations through creation of noncovalent aromatic macrocycles in which adjacent aromatic segments can slide with respect to one another in response to external triggers. The noncovalent macrocycles spontaneously stack on top of each other to form a tubular struc-

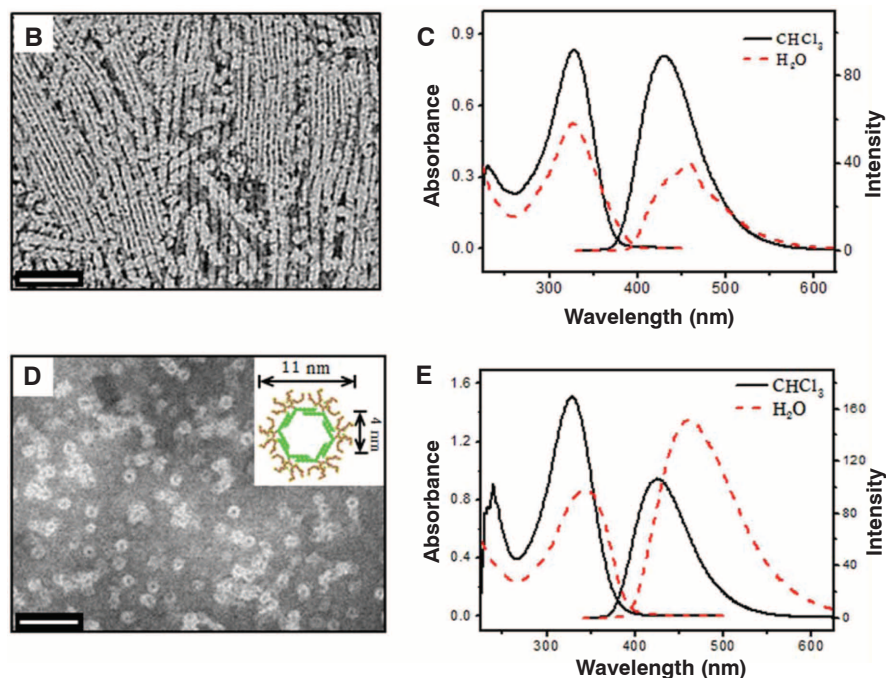
ture with a hydrophobic interior in aqueous solution. The resulting tubules undergo a drastic contraction of ~50% in internal volume upon heating, with chirality inversion, through the sliding motion between the neighboring aromatic segments within the macrocycles. The aromatic interior of the tubules can encapsulate fullerene molecules during assembly, some of which are released upon contraction by heating.

The self-assembling molecules that form this aggregate consist of a bent-shaped aromatic segment with an internal angle of 120° and a hydrophilic oligoether dendron grafted at its apex (Fig. 1A). Molecule **1** self-assembles into the hollow tubules in dilute aqueous solutions. Transmission electron microscopy (TEM) showed elongated objects with an external diameter of 7 nm and a hollow interior with a diameter of 3 nm (Fig. 1B). A few toroids, albeit rare, could be observed in the images. The diameters of the toroids are identical to those of the tubules, suggesting that the tubules originated from toroidal stacking. Dy-

namic light scattering (DLS) experiments with a 0.002 weight percent (wt %) aqueous solution of **1** showed the concentration-independent hydrodynamic radius to be ~145 nm, which is consistent with the TEM results (fig. S2, A and B). Optical spectra of **1** displayed a blue-shifted absorption maximum and reduced fluorescence intensity in water compared with chloroform solutions, indicative of *H*-type stacking of the aromatic segments (Fig. 1C). Circular dichroism (CD) spectra of the aqueous solutions showed a significant Cotton effect above certain concentrations (0.002 wt %) in the spectral range of the aromatic units, indicating that the tubules adopt a one-handed helical structure (fig. S2C). To gain insight into the primary structure of the aggregates, we conducted vapor pressure osmometry (VPO) measurements in dioxane in the concentration range of 12 to 35 g/kg (sample/solvent). The molecular weight of the primary aggregate was measured to be 10.5 kD, six times as large as that of the single molecule (1841 D) (fig. S2D).



**Fig. 1.** (A) Molecular structure of bent-shaped rod amphiphiles. (B) A TEM image of **1** from 0.01 wt % aqueous solution (scale bar, 50 nm). (C) Absorption and emission spectra of **1** in  $\text{CHCl}_3$  (black and solid line) and in aqueous solution (red and dashed line). (D) A TEM image of **2a** from 0.002 wt % aqueous solution (scale bar, 50 nm). (E) Absorption and emission spectra of **2a** in  $\text{CHCl}_3$  (black and solid line) and in aqueous solution (red and dashed line).



These observations, together with inspection of CPK models, indicate that **1** self-assembles via a fully overlapped packing arrangement into the hexameric macrocycles, which, in turn, stack on top of each other with mutual rotation in a single direction to form helical tubules.

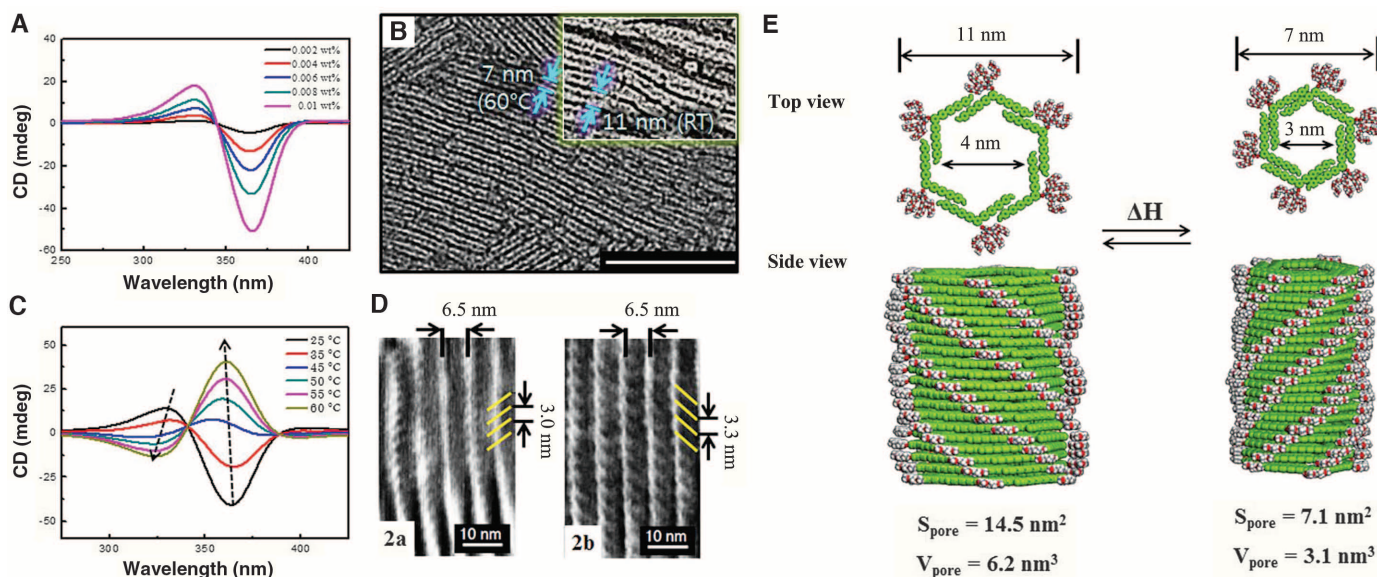
We envisioned that introduction of a pyridine unit on the concave side of the apex of the bent-shaped aromatic segment might induce adjacent molecules to slide into a looser packing arrangement because pyridine is well-known to form water clusters through hydrogen bonding (18, 19). In this context, we prepared **2a** containing a pyridine unit at its valley position. DLS experiments with dilute aqueous solutions (0.002 wt %) indicated formation of discrete aggregates with a diameter of ~12 nm (fig. S3A). The aggregate structure was visualized by TEM (Fig. 1D). When the sample was cast from the solution and then negatively stained with uranyl acetate, the image showed toroidal objects with a uniform diameter of 11 nm and an internal cavity 4 nm in diameter. To further confirm the formation of the toroids, we performed atomic force microscopy (AFM) measurements of the samples prepared by drop casting of the aqueous solution (0.002 wt %) on a mica surface (fig. S3C). The image clearly revealed toroidal objects 3.4 Å in height, demonstrating that the toroidal objects are single stacks of the hexameric macrocycles. In great contrast to **1**, the absorption maximum of **2a** in water is red-shifted and the fluorescence intensity apparently enhanced with respect to those observed in chloroform solutions, indicative of *J*-type stacking of the aromatic segments (Fig. 1E) (20). All these observations indicate that **2a** self-assembles into expanded hexameric macrocycles through a

slipped packing arrangement between the aromatic segments. The expansion of the noncovalent macrocycles is reflected in increased dimensions of both external and internal diameters compared with that of **1** (Fig. 1D). This expansion of the hexameric macrocycles could be explained by considering the formation of water clusters at the pyridine unit of the *v*-position of **2a**. To corroborate the formation of water clusters at the pyridine unit, we prepared model compound **7a** (2,6-dibromo pyridine derivative). Proton nuclear magnetic resonance (<sup>1</sup>H-NMR) measurements of **7a** showed that the proton signals associated with the pyridine are downfield-shifted when the solvent is changed from CDCl<sub>3</sub> to D<sub>2</sub>O/H<sub>2</sub>O, indicating the formation of hydrogen bonds with H<sub>2</sub>O at the pyridine unit (fig. S3E). The water cluster enforces slipping of adjacent aromatic segments into a looser packing arrangement to reduce steric crowding at the valley position of the internal cavity. The sliding motion between the adjacent aromatic segments gives rise to the formation of the expanded hexameric macrocycles with an increase in external diameter from 7 to 11 nm.

The expanded macrocycles undergo supramolecular polymerization with increasing concentration by stacking on top of each other with mutual rotation in the same direction to form elongated helical tubules. DLS measurements showed that hydrodynamic radius increases, with increasing concentration within this range of concentration (fig. S4A). The TEM image of the solution with a concentration of 0.02 wt % revealed the formation of elongated tubules with an external diameter of 11 nm and internal diameter of 4 nm (Fig. 2B, fig. S5A). These results demon-

strate that the diameters of the hexameric macrocycles are conserved even after supramolecular polymerization. This is also reflected in the unaltered optical spectroscopic features after transformation into the tubules; that is, preservation of the slipped packing arrangement of the aromatic segments (fig. S4C). CD spectra show increased intensity with increasing concentration, indicative of helical stacking of the macrocycles with a preferred handedness (Fig. 2A). Assemblies derived from the enantiomer **2b** display opposite CD signals with a mirror-image relationship (fig. S4D), indicating that the molecular chirality is transferred to the self-assembled structure (21, 22).

The formation of hollow tubules with oligoether dendritic exterior and pyridine interior suggested that aqueous solutions of **2a** would exhibit thermoresponsive behavior, because the ethylene oxide chains and the pyridine units undergo thermally regulated dehydration upon heating (23). This dehydration was confirmed by temperature-dependent <sup>1</sup>H-NMR measurements with a reference **7a** (fig. S5C). The resonances associated with pyridine units shifted upfield, and those of the ethylene oxide chains broadened, together with a decrease in intensity upon heating above 55°C, clearly demonstrating the loss of hydrogen bonding interactions between pyridine nitrogens or ether oxygens and water molecules. The dehydration process could allow sliding of the aromatic segments from the slipped arrangement into the fully overlapped motif to maximize aromatic  $\pi$ - $\pi$  stacking interactions. This packing consideration is reflected in the blue-shifted absorption maximum and fluorescence quenching upon heating (fig. S6, A and C). We believe



**Fig. 2.** (A) CD spectra of **2a** in aqueous solution at various concentrations. (B) TEM image of **2a** from 0.01 wt % aqueous solution prepared at 60°C (scale bar, 100 nm) (Inset) Prepared at room temperature. (C) Temperature-dependent CD spectra of **2a** (0.01 wt %) in aqueous solution. (D) AFM phase images (scale = 60 by 35 nm) of 2D self-assembled right-

handed **2a** (left) and left-handed **2b** (right) on HOPG. (E) Schematic representation of reversible switching of the tubules between expanded and contracted states with chirality inversion.  $S_{\text{pore}}$ , cross-sectional area of hollow pore in a slice of the macrocycles;  $V_{\text{pore}}$ , volume of hollow pore in a slice of the macrocycles).



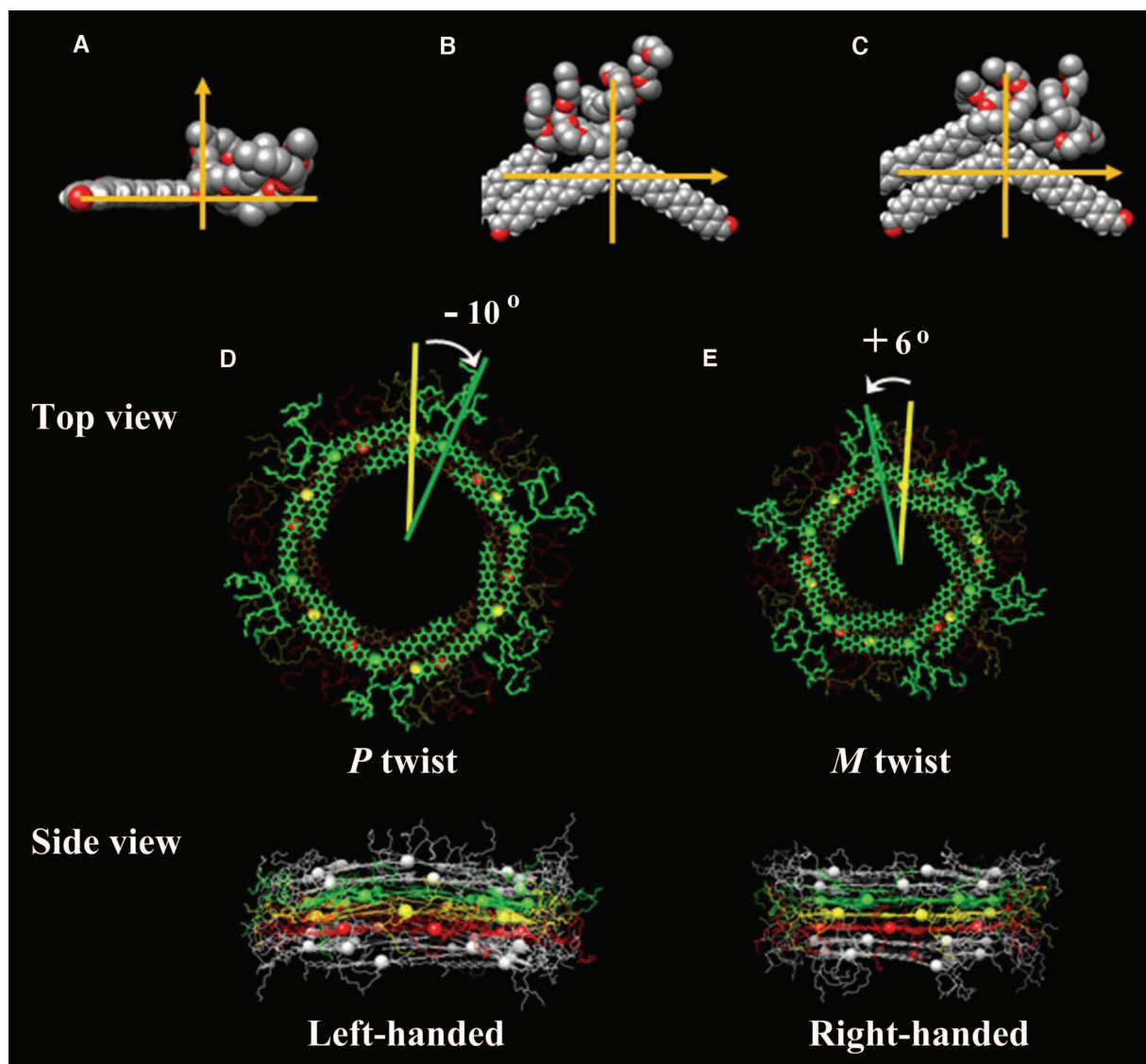
that the observed spectral changes are the result of a fully overlapped *H*-type packing arrangement of the aromatic segments (20). To confirm this molecular rearrangement upon heating of the tubular structure, we carried out TEM experiments. On heating to 60°C, the tubular structure was retained (Fig. 2B). However, the diameter drastically decreased upon heating. The density profile taken perpendicular to the long axis of the tubule showed the external and internal diameters to be 7 and 3 nm, respectively, indicative of a 47% reduction in cross-sectional area of the internal cavity with respect to that at room temperature (fig. S5, A and B). This size change, together with all the spectral changes, is fully reversible on cooling and subsequent heating cycles. We conclude from these experimental results that this reversible contraction of the tubules results from the molecular rearrangement through

a sliding motion between the adjacent aromatic segments.

Notably, this expansion-contraction motion of the tubules is accompanied by chirality inversion (Fig. 2C). The CD spectrum of **2a** showed a strong signal with a negative Cotton effect at 369 nm, the absorption wavelength of the aromatic unit, indicating the formation of a helical superstructure with a preferred handedness. Upon heating, however, the CD signal was inverted from the negative minimum to a strong positive Cotton effect, indicating that the helical sense switches to opposite-handedness (24, 25). The CD signal with a negative minimum decreases gradually up to 45°C and is completely reversed to a positive Cotton effect upon further heating. The maximum wavelength was blue-shifted, and the maximum intensity of the CD signal was identical but with an opposite Cotton effect from

the room temperature signal, demonstrating that the tubules have highly dynamic helicities in response to temperature (fig. S7A). This positive Cotton effect is the same as that in the temperature-independent CD signal of **1**, suggesting that the tubule of **2a** at higher temperatures adopts the same sense of helicity as **1** (fig. S7C). This result demonstrates that the hexameric macrocycles stack in the identical helical sense when they are based on fully overlapped arrangements of the aromatic segments (*H*-type packing).

To corroborate the handedness of the helical tubules, we performed AFM experiments with **2a** and **2b** on highly oriented pyrolytic graphite (HOPG) in the completely dried state. The images revealed bundles of rod-like aggregates with a diameter of ~7 nm (fig. S8), suggesting that the rods on the HOPG surface correspond to the contracted tubules derived from the *H*-type aggre-



**Fig. 3.** Molecular dynamics simulations. (A to C) Orientation of the dendritic coil in (A) the helical axis plane, (B) the macrocyclic plane of the expanded state, and (C) the macrocyclic plane of the contracted state. (D and E) Rep-

resentative images of top and side views of the (D) expanded and (E) contracted forms of helical tubules obtained from the simulations. Pyridine nitrogen atoms are represented as colored spheres.



gation in aqueous solution. Indeed, the solution CD signals observed at room temperature inverted to opposite signs upon complete drying (fig. S9). The magnified image of tubules comprising **2a** (Fig. 2D, left) revealed a right-handed helical structure with a pitch of 3 nm that is the mirror image of the tubules comprising **2b** (Fig. 2D, right). This mirror image relationship is consistent with the CD results. In contrast with contracted tubules on HOPG, elongated objects with a diameter of 11 to 12 nm probably corresponding to the expanded state (*J*-type packing) were observed when aqueous solutions of **2a** and **2b** were cast on hydrophilic mica substrates. Although we had difficulty observing clean helical structures on the objects, most likely due to their soft and hydrated surfaces, the mirror-image helices formed through the enantiomers in the expanded state were also observed in part (fig. S10, C and D).

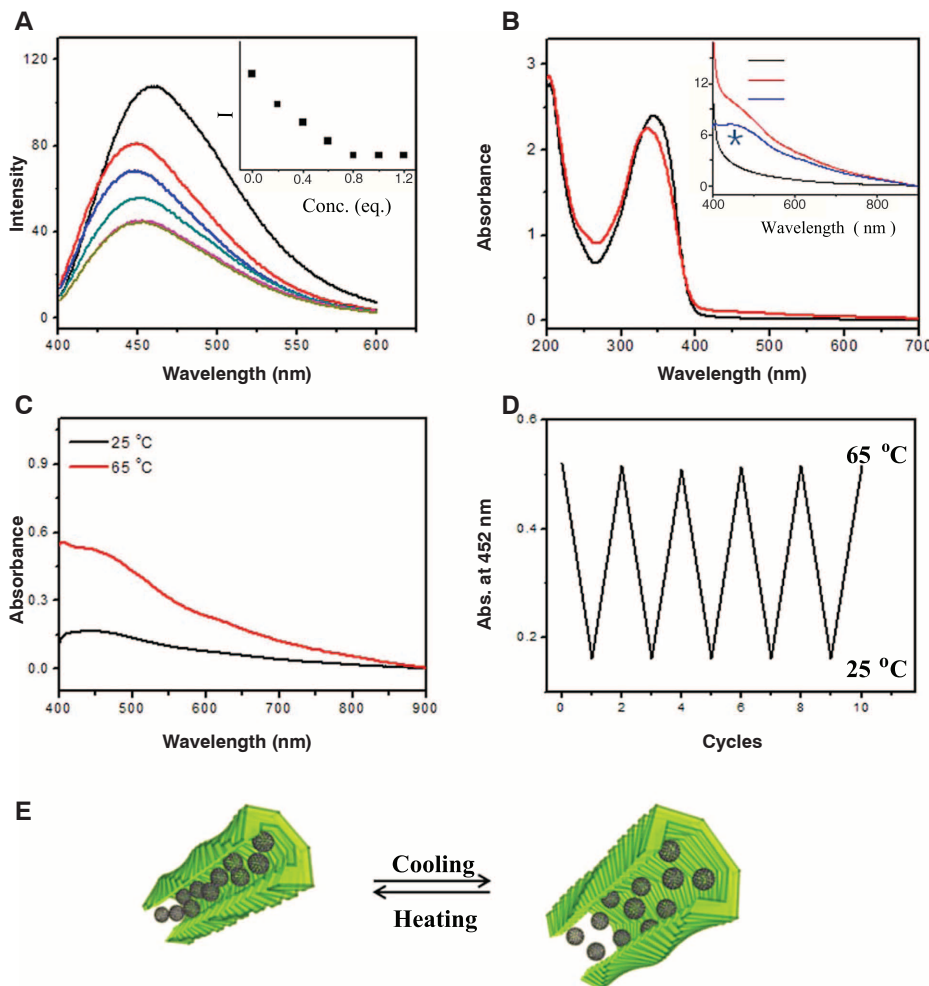
Molecular dynamics simulations of **2a** using the GROMACS 4 program supported attribution of the chirality inversion to an orientational change with temperature of the dendritic chains on the tubule exterior (Fig. 3). According to the calculations, the expanded macrocycles stack on top of each other with mutual rotations at an angle of  $-10^\circ$  in the same direction to give

rise to left-handed helical tubules, whereas the contracted macrocycles twist  $+6^\circ$  to give right-handedness (Fig. 3). The observed inversion of helical handedness seems to result from two independent orientations of the dendritic moieties with respect to both the helical axis and macrocycle plane (fig. S11). Due to the chirality of the ethylene oxide chain, the oligoether dendron adopts an upward orientation irrespective of the expanded or contracted motif (Fig. 3A). However, the dendritic segments adopt different orientations with variation of the diameter of the helical tubule along the macrocycle plane. In the expanded motif, there are marginal spaces outside of the aromatic rings that the dendrimers occupy, resulting in steric repulsions between bulky dendritic segments on the left side of each vertex (Fig. 3B). To relieve the steric repulsions without sacrificing  $\pi$ -stacking interactions between the rods, the upper-layered macrocycles rotate clockwise to form left-handed helical tubules (Fig. 3D). However, in the contracted motif, the closely stacked macrocycles crowd out the dendrimers from the marginal space leading to steric crowding on the right side of each vertex (Fig. 3C). This spatial requirement induces the upper-layered macrocycles to rotate

counter clockwise, giving rise to right-handed helical tubules (Fig. 3E).

This dynamic motion of the tubules can be accompanied by packing variations of encapsulated hydrophobic guest molecules (Fig. 4). The tubules with aromatic cavities can take up  $C_{60}$  molecules through hydrophobic interactions during assembly. Upon addition of  $C_{60}$  to the solution of **2a**, the fluorescence intensity was considerably suppressed (Fig. 4A), indicating that  $C_{60}$  was effectively encapsulated within the hydrophobic interior of the tubules (26, 27). The fluorescence intensity decreased with an increase in fullerene content up to a certain point [0.8 equivalent (equiv.)], beyond which the fluorescence did not change upon further fullerene addition to the solution (Fig. 4A). Therefore, the maximum amount of  $C_{60}$  loading per bent-shaped molecule can be considered 0.8 equiv. On heating, a portion of the  $C_{60}$  guest population was released from the tubular interior due to the shrinkage of the tubules. The absorption intensity corresponding to  $C_{60}$  decreased to 0.4 equiv., indicating that 0.4 equiv. of  $C_{60}$  escape from the tubular interior through contraction (fig. S12). Subsequent cooling of the supernatant to room temperature led to a color change from dark to pale yellow, with considerably

**Fig. 4.** Encapsulation of fullerenes within the tubular cavities. **(A)** Fluorescence spectra ( $\lambda_{\text{ex}} = 380$  nm) of **2a** in aqueous solution (0.05 wt %) without  $C_{60}$  and with 0.1 to 1.2 equiv.  $C_{60}$ . The inset shows the variation of emission intensity at 450 nm. **(B)** Ultraviolet-visible (UV-vis) spectra of **2a** and **2a**+ $C_{60}$  in aqueous solution. The inset shows 10 $\times$  magnified visible region. **(C)** UV-vis spectra of **2a** in aqueous solution with 0.4 equiv.  $C_{60}$ . **(D)** Reversible absorbance change of  $C_{60}$  inside tubular cavity upon temperature variation. **(E)** Schematic representation of the regulation of  $C_{60}$ - $C_{60}$  interactions within the tubular cavities when the tubule contains 0.4 equiv of  $C_{60}$ .



reduced absorbance at 452 nm (Fig. 4C), indicating that  $C_{60}$ - $C_{60}$  interactions are diminished due to expansion of the internal volume (28). The absorbance and solution color immediately recover to those of the contracted state upon subsequent heating, indicating that the breathing motion of the tubules leads to a reversible switch between tight and loose packing of the fullerenes within the tubular cavity (Fig. 4, D and E).

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## Supplementary Materials

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Materials and Methods  
Supplementary Text  
Figs. S1 to S12  
References (29–37)

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# A Crystalline Singlet Phosphinonitrene: A Nitrogen Atom–Transfer Agent

Fabian Dielmann,<sup>1,2</sup> Olivier Back,<sup>1</sup> Martin Henry-Ellinger,<sup>1,2</sup> Paul Jerabek,<sup>3</sup> Gernot Frenking,<sup>3</sup> Guy Bertrand<sup>1,2\*</sup>

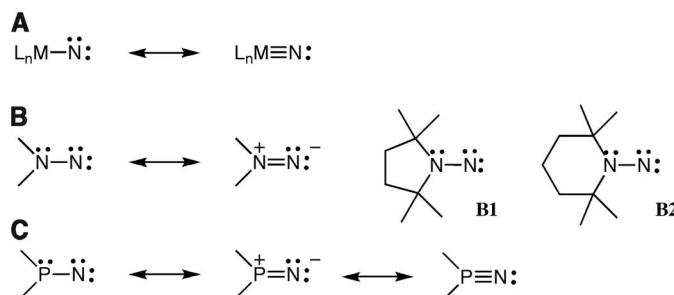
A variety of transition metal–nitrido complexes (metallonitrenes) have been isolated and studied in the context of modeling intermediates in biological nitrogen fixation by the nitrogenase enzymes and the industrial Haber-Bosch hydrogenation of nitrogen gas into ammonia. In contrast, nonmetallic nitrenes have so far only been spectroscopically observed at low temperatures, despite their intermediacy in a range of organic reactions. Here, we report the synthesis of a bis(imidazolidin-2-iminato)phosphinonitrene, which is stable at room temperature in solution and can even be isolated in the solid state. The bonding between phosphorus and nitrogen is analogous to that observed for metallonitrenes. We also show that this nitrido phosphorus derivative can be used to transfer a nitrogen atom to organic fragments, a difficult task for transition metal–nitrido complexes.

Reactive intermediates play a central role in modern chemistry (1). The isolation of stable analogs has facilitated a better understanding of the reaction mechanisms and, in some cases, has given rise to distinct applications, as illustrated by the recent developments in carbene chemistry (2–7). However, there are still many families of reactive intermediates that

have eluded the synthetic skills of investigators: Among these are nitrenes (8, 9), the nitrogen analogs of carbenes, which are compounds with a neutral monocoordinate nitrogen atom featuring either a lone pair and two singly occupied non-

bonding orbitals (a triplet state) or two lone pairs and an accessible vacant orbital (a singlet state). These highly reactive electron-deficient species are involved in many reactions of synthetic interest such as CH-insertion, ring expansion, and aziridination processes but have never been isolated, with the exception of metallonitrenes **A** (Fig. 1A) (10–14). The latter can also be regarded as transition metal–nitrido complexes ( $L_nMN$ , where  $L$  is a ligand, and  $M$  is a metal), because one of their contributing resonance structures features a metal–nitrogen multiple bond. An  $M-N$   $\sigma$  bond is formed from the interaction between an orbital of  $\sigma$  symmetry on the metal and a  $p_z$  or  $sp$ -hybrid orbital of the nitrogen atom. In addition,  $\pi$  bonds can be formed via overlap of the nitrogen  $p_x$  and  $p_y$  orbitals with appropriate orbitals of  $\pi$  symmetry on the metal. Overall, a metal–nitrogen bond order of three is possible.

The most stable nonmetallic nitrenes are the aminonitrenes **B** (Fig. 1B). The *N*-(2,2,5,5-tetramethylpyrrolidyl)nitrene (**B1**) and *N*-(2,2,6,6-tetramethylpiperidyl)nitrene (**B2**) (Fig. 1B) discovered by Dervan and co-workers (15–18) are sufficiently long lived in solution at  $-78^\circ\text{C}$  to permit spectroscopic characterization and pu-



**Fig. 1.** Metallo- (**A**), amino- (**B**), and phosphino- (**C**) nitrenes (left) and their respective metal nitrido, 1,1-diazene, and phosphorus nitrido resonance forms (right). Aminonitrenes **B1** and **B2** are stable at low temperature.

<sup>1</sup>University of California Riverside–CNRS Joint Research Chemistry Laboratory (UMI 2957), Department of Chemistry, University of California, Riverside, CA 92521–0403, USA. <sup>2</sup>University of California San Diego–CNRS Joint Research Chemistry Laboratory (UMI 3555), Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA 92093–0343, USA. <sup>3</sup>Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany.

\*To whom correspondence should be addressed. E-mail: guybertrand@ucsd.edu

rification by low-temperature chromatography ( $-88^{\circ}\text{C}$ ). The relative stability of aminonitrenes has been attributed to the interaction of the amino-N lone pair with a vacant orbital of the nitrene, as shown by the 1,1-diazene resonance form, a mode of stabilization widely applied for carbenes (19, 20).

We reasoned that replacing the amino group of **B** (Fig. 1B) by a phosphino group should further enhance the stabilization of the nitrene because of the possible back-donation of a nitrene lone pair into an accessible  $\sigma^*$  orbital at phosphorus, as manifested in the nitrido phosphorus resonance form (Fig. 1C) (21–27). Here, we report the synthesis and single-crystal x-ray diffraction study of a phosphinonitrene, which is stable for days at room temperature (both in solution and in the solid state) but remains very reactive, as shown by its 1,1-coupling reaction with an isonitrile.

In 2001, a computational study by Schoeller and Rozhenko (23) concluded that the most appropriate substituents for stabilizing phosphinonitrenes should be strong  $\pi$  donors and weak  $\sigma$  acceptors. Schoeller and Rozhenko suggested that “substituents, which bear an imino function in the  $\alpha$ -position to the phosphorus atom, e.g., the phosphaniminato group” would be the best choice (23). Based on this prediction and on the efficiency of the imidazolidin-2-iminato group for the stabilization of various phosphorus (28) and metallic species (29–31), we prepared azide **2** (32) from the corresponding chloride derivative **1** ( $\text{X} = \text{Cl}$ ) (Fig. 2) (28).

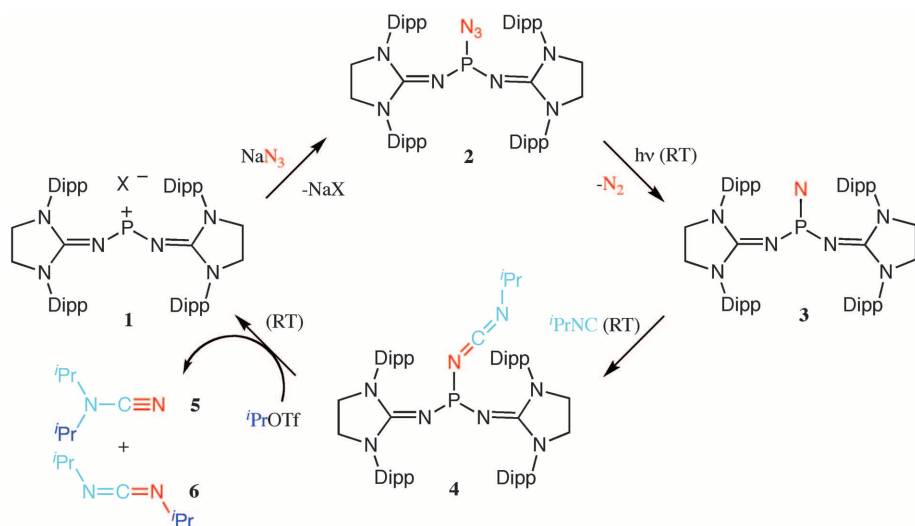
We stirred a toluene solution of **2** under irradiation (254 nm) at room temperature for 2 hours in a quartz tube. After removing the solvent under reduced pressure, we obtained nitrene **3** as a pale yellow solid in 92% yield. The  $^{31}\text{P}$  nuclear

magnetic resonance (NMR) spectrum shows a singlet at 7.7 parts per million (ppm), shifted upfield in comparison with the azide starting material **2** (111.0 ppm), indicative of a hypervalent phosphorus center. To measure the  $^{15}\text{N}$  NMR chemical shift of the nitrene nitrogen (33), we prepared the  $^{15}\text{N}$ -enriched azide **2\*** ( $\text{R}_2\text{P}^{15}\text{NN}^{15}\text{N}$ ) from **1** and  $\text{Na}^{15}\text{NNN}$  and photolyzed it to form labeled **3\***. For **2\***, the  $\text{N}_\gamma$  and  $\text{N}_\alpha$  positions exhibit a singlet at 196.6 ppm and a doublet at 95.8 ppm (coupling constant  $J_{\text{PN}} = 103$  Hz), respectively. The nitrene nitrogen of **3\*** appears as a doublet with a larger PN coupling constant (144 Hz), which again suggests the presence of a PN multiple bond. This signal is downfield (266.0 ppm) compared with that of **2\*** but is strongly shielded compared with the resonance observed for the nitrene nitrogen of *N*-(2,2,6,6-tetramethylpiperidyl)nitrene **B1** (917.0 ppm) (Fig. 1B) (18). The same high-field shielding effect is observed when comparing the  $^{13}\text{C}$  chemical shift of the carbene carbon of phosphinocarbenes (80 to 150 ppm) with that of aminocarbenes (200 to 400 ppm) (34).

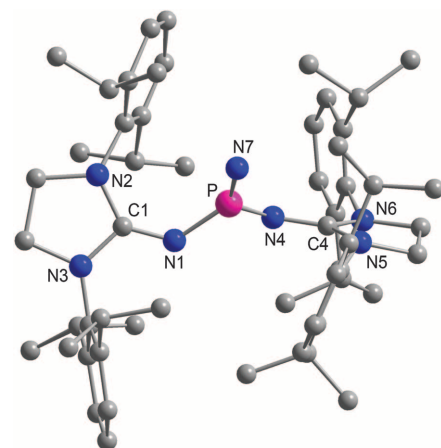
We obtained single crystals of **3** by dissolving the powder in warm hexane, filtering, and storing the filtrate at  $-30^{\circ}\text{C}$ . An x-ray diffraction study (Fig. 3) shows that the phosphorus atom is in a planar environment (sum of the angles:  $359.9^{\circ}$ ), and the P–N(7) bond length is very short [ $1.457(8)$  Å, number in parentheses indicates the standard uncertainty]. For comparison, the P–N(1) and P–N(4) bond distances are  $1.629(8)$  and  $1.618(8)$  Å, respectively, and reported PN double bonds are in the range of 1.50 to 1.60 Å (35). All of these geometric parameters suggest the presence of a PN multiple bond.

To gain further insight into the electronic structure of **3**, we used quantum chemical methods to

study the model compound **3<sub>model</sub>** (which has a phenyl group in place of the 2,6-diisopropylphenyl substituents). At the M05-2X/TZVPP level of theory, the triplet state features a nonplanar phosphorus center and is 36.0 kcal/mol higher in energy than the singlet state. For the latter, the geometry is in good agreement with the experimental data (see fig. S21 and accompanying text). Natural bond orbital (NBO) calculations give large negative partial charges for N7 ( $-1.22e$ , where  $e$  is the elementary charge) and N1 and N4 ( $-0.96e$ ), whereas the phosphorus atom carries a large positive charge ( $+1.92e$ ). There is a P–N7  $\sigma$  bond and an out-of-plane  $\pi_\perp$  bond, both of which are clearly polarized toward the nitrogen atom; the latter has one  $\sigma$  and one in-plane  $\pi_\parallel$  lone-pair orbital. The Wiberg bond order values suggest a double bond for P–N7 (2.09) and single bonds for P–N1 and P–N4 (0.85). The NBO results are supported by energy decomposition analysis with natural orbitals for chemical valence (EDA-NOCV) calculations of nitrene **3<sub>model</sub>**, using two different fragmentation schemes. The P–N7 bond was broken (i) into neutral fragments (nitrogen atom in the  $^2\text{P}$  reference state and the phosphinyl fragment in the matching doublet state) and (ii) into charged fragments ( $\text{N}^-$  and the phosphinyl cation in the triplet state). In both cases, the results show that the P–N7 covalent bond comes mainly from the  $\sigma$  bond (52% for the neutral fragments, 47% for the charged fragments) and from the  $\pi_\perp$  bond (36% for the neutral fragments, 33% for the charged fragments). The contribution of the in-plane  $\pi_\parallel$  interactions is much smaller (8% for the neutral fragments, 11% for the charged fragments). Figure 4, A to C, shows the highest-lying occupied molecular orbitals of **3<sub>model</sub>** (with a methyl group in place of the 2,6-diisopropylphenyl

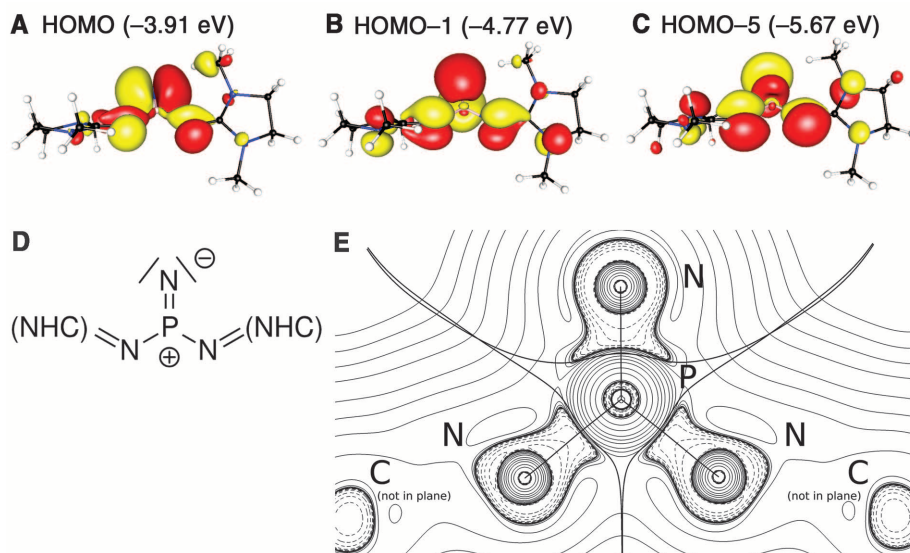


**Fig. 2.** Synthesis of the room temperature (RT), stable phosphinonitrene **3** (Dipp, 2,6-diisopropylphenyl). Similarly to metallonitrenes, **3** reacts with isopropyl isocyanide, affording carbodiimide **4**. Addition of isopropyltriflate completes the nitrogen atom-transfer synthetic cycle, giving back the starting phosphonium salt **1** ( $\text{X} = \text{OTf}$ ) and a mixture of cyanamide **5** and carbodiimide **6**. hv, irradiation (254 nm);  $^i\text{Pr}$ , isopropyl group.



**Fig. 3.** Molecular view of phosphinonitrene **3** in the solid state (for clarity, H atoms are omitted). Selected bond lengths and angles: C1–N1,  $1.282 \pm 11$  Å; C1–N2,  $1.370 \pm 11$  Å; C1–N3,  $1.374 \pm 11$  Å; C4–N4,  $1.312 \pm 11$  Å; C4–N5,  $1.368 \pm 11$  Å; C4–N6,  $1.381 \pm 11$  Å; P–N1,  $1.629 \pm 8$  Å; P–N4,  $1.618 \pm 8$  Å; P–N7,  $1.457 \pm 8$  Å; C1–N1–P,  $126.7 \pm 7^{\circ}$ ; C4–N4–P,  $127.2 \pm 6^{\circ}$ ; N1–P–N4,  $101.8 \pm 4^{\circ}$ ; N1–P–N7,  $129.5 \pm 5^{\circ}$ ; and N4–P–N7,  $128.6 \pm 5^{\circ}$ .





**Fig. 4.** (A to C) Plot of the calculated highest-lying occupied molecular orbitals of the phosphininonitrene **3<sub>model</sub>** (with a methyl group in place of the 2,6-diisopropylphenyl substituents) exhibiting P-N bonding. (D) Lewis structure for **3<sub>model</sub>** (with a phenyl group in place of the 2,6-diisopropylphenyl substituents), as suggested by the analysis of the bonding situation. (E) Laplacian distribution  $\nabla^2\rho(r)$  of nitrene **3<sub>model</sub>** in the central NNPN plane, showing the polarization of the P-N bonds toward the nitrogen atom.

substituents for clarity), which complies with the NBO and EDA-NOCV results. The highest occupied molecular orbital (HOMO) is the in-plane  $\pi_{\parallel}$  lone-pair orbital at N7, whereas the HOMO-1 is the polarized out-of-plane P-N7  $\pi_{\perp}$  orbital. The HOMO-5 is mainly the P-N7  $\sigma$ -bonding orbital. In general, no 1:1 correspondence exists between the delocalized canonical molecular orbitals and the localized Lewis structure, which is shown in Fig. 4D. The polarization of the P-N bonds toward the nitrogen end becomes clearly visible from the Laplacian distribution  $\nabla^2\rho(r)$  of the nitrene in the central NNPN plane of **3<sub>model</sub>** (Fig. 4E).

The analogy between phosphininonitrene **3** and metallonitrenes **A** (Fig. 1A) prompted us to investigate the possibility of using **3** as a nitrogen atom-transfer agent. The formation of N-C bonds by two-electron nitrogen atom transfer from metal nitrido complexes to a variety of organic substrates has been reported (36–38), but apart from two exceptions (39, 40), the functionalized substrates remain bound to the metal. As a proof of concept, we added isopropyl isonitrile to nitrene **3** at room temperature and obtained carbodiimide **4** in 81% yield (according to  $^{31}\text{P}$  NMR) (Fig. 2). To achieve a synthetic cycle, a difficult task in the case of metal complexes **A** (Fig. 1A) (39), we treated carbodiimide **4** with isopropyl trifluoromethanesulfonate (PrOTf). We recovered phosphonium salt **1** (TfO<sup>−</sup> as counteranion) in 86% yield and a mixture of cyanamide **5** and carbodiimide **6** in 42 and 8% yields, respectively (Fig. 2).

More than two decades after the discovery of a stable carbene—namely, a phosphinocarbene (41, 42)—this work shows that nitrenes can be isolated as well. Phosphininonitrenes feature a

PN multiple bond and are the monomers corresponding to the polyphosphazenes, a well-known family of polymers (43, 44). The complete nitrogen atom transfer from the phosphininonitrene is another demonstration that main-group compounds, and especially electron-deficient species, can mimic the chemical behavior of transition metals (6, 45–48).

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#### Supplementary Materials

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Supplementary Text  
Figs. S1 to S22  
Table S1  
References (49–60)

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# A Fully Size-Resolved Perspective on the Crystallization of Water Clusters

Christoph C. Pradzynski,<sup>1</sup> Richard M. Forck,<sup>1</sup> Thomas Zeuch,<sup>1\*</sup> Petr Slavičák,<sup>2</sup> Udo Buck<sup>3\*</sup>

The number of water molecules needed to form the smallest ice crystals has proven challenging to pinpoint experimentally. This information would help to better understand the hydrogen-bonding interactions that account for the macroscopic properties of water. Here, we report infrared (IR) spectra of precisely size-selected  $(\text{H}_2\text{O})_n$  clusters, with  $n$  ranging from 85 to 475; sodium doping and associated IR excitation–modulated photoionization spectroscopy allowed the study of this previously intractable size domain. Spectral features indicating the onset of crystallization are first observed for  $n = 275 \pm 25$ ; for  $n = 475 \pm 25$ , the well-known band of crystalline ice around  $3200\text{ cm}^{-1}$  dominates the OH-stretching region. The applied method has the potential to push size-resolved IR spectroscopy of neutral clusters more broadly to the 100- to 1000-molecule range, in which many solvents start to manifest condensed phase properties.

A central aim of cluster science is to unravel the microscopic behavior of the condensed phase. A special attraction originates from the subtle features of the hydrogen-bonding interactions that govern the bulk and surface properties of ice. Water clusters themselves also play an important role in atmospheric science and interstellar media (1, 2). The nucleation and growth of nanoparticles (1, 3) and chemical reactions at the interface of ocean-spray aerosols (4) are just two examples among many others. The structures of small  $(\text{H}_2\text{O})_n$  clusters ( $n = 2$  to 10) and of ice nanoparticles have been quite well characterized experimentally and theoretically [(5, 6) and references therein]. In contrast,

the size range from  $n = 100$  to 1000, in which the crystallization of water is assumed to begin, has been inaccessible to precisely size-selective techniques.

Deflection of clusters in a scattering experiment with an atomic beam can finely resolve size by specifying the angle and the velocity after the collision (7). Vibrational predissociation is then applied to obtain the infrared (IR) spectrum of the size-selected cluster. However, this method has only proven applicable to sizes up to  $n = 12$  (5, 8, 9). Similar results in this size range have been obtained by other methods that are based on rotational resolution (10), argon tagging (11), or the addition of aromatic molecules and their detection through ultraviolet (UV) excitation (12–14). The latter method has also been applied to water cluster sizes up to  $n = 50$  by adding phenol (15, 16), but the interpretation of the results can be hampered by fragmentation in the detection process.

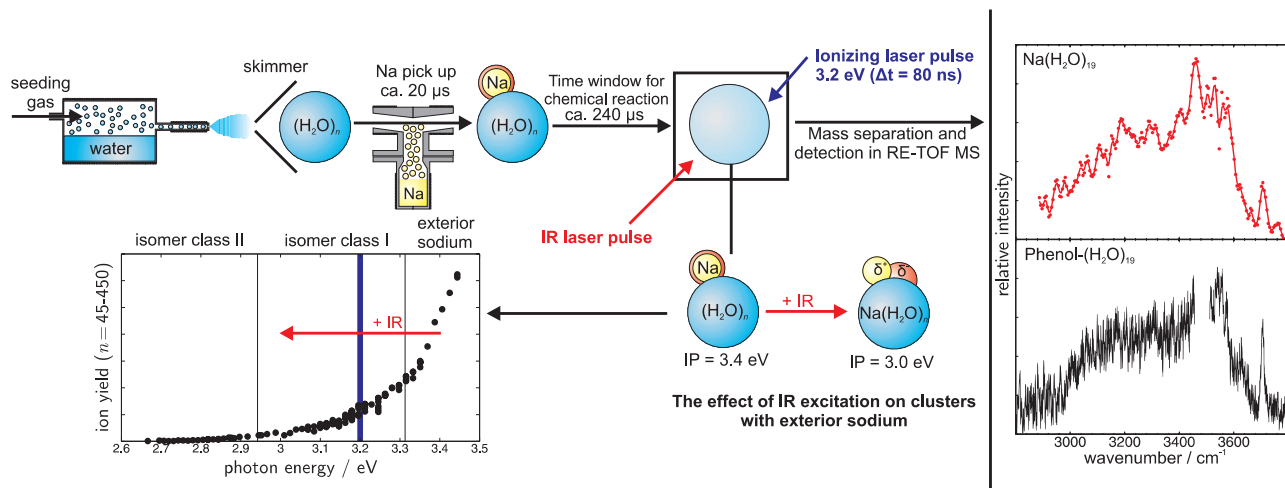
This effect can be avoided by using a sodium (Na) atom as dopant on the cluster. Because of its

low ionization potential (17, 18), easily available, tunable UV lasers can eject in a one-photon absorption process photoelectrons from the corresponding clusters close to the threshold without inducing fragmentation. Buck and co-workers first applied this method to precisely determine cluster sizes for ammonia, water, and argon (Ar) up to  $n = 10,000$ . The measured size distributions follow for large clusters a rather broad log-normal distribution. Using this method, the evaporation—caused by electron bombardment ionization—was revealed (19, 20). IR spectra for cluster distributions of average sizes up to  $\langle n \rangle = 1000$  were measured with fragment detection, which exhibits, in addition to the present size distribution, a special sensitivity to the surface (6, 21).

These limitations do not apply to a type of IR spectroscopy that uses the Na doping method introduced by Steinbach and Buck (22). IR irradiation of the clusters before photoionization enhances their photoionization probability when the UV wavelength is tuned to energies below the threshold. The resulting signal enhancement upon IR excitation enabled fully size-resolved IR spectral measurements for  $\text{Na}(\text{H}_2\text{O})_n$  clusters up to  $n = 60$  (22, 23). Recent studies succeeded in giving the important hints for unravelling the action effect that is exploited for generating IR spectra by using this technique (24, 25). As we show here, this turned out to be the key for developing an infrared excitation/single-photon ionization technique that augments the size-selectivity far beyond  $n = 100$  and that is for large cluster sizes undisturbed by effects of the Na atom. This allows us for the first time to measure fully size-resolved IR spectra and to identify the onset of water crystallization on the basis of the different peaks in the IR spectrum. The particular suitability of this criterion is the considerable shift between the absorption maxima. Only crystalline water has a lower lying absorption maximum in the OH stretch region around  $3200\text{ cm}^{-1}$ ; for amorphous

<sup>1</sup>Institut für Physikalische Chemie, Universität Göttingen, Tammannstr. 6, D-37077 Göttingen, Germany. <sup>2</sup>Department of Physical Chemistry, Institute of Chemical Technology Prague, Technická 5, 16628 Prague 6, Czech Republic. <sup>3</sup>Max-Planck-Institut für Dynamik und Selbstorganisation, Am Faßberg 17, D-37077 Göttingen, Germany.

\*To whom correspondence should be addressed. E-mail: tzeuch1@gwdg.de (T.Z.); ubuck@gwdg.de (U.B.)



**Fig. 1.** Photoionization scheme, origin of action effect and (right) comparison of IR spectra of Na doped with phenol-doped  $(\text{H}_2\text{O})_{19}$  clusters. The bottom trace corresponds to the phenol-water cluster [reprinted with permission from

(16), copyright (2011) American Chemical Society]; the top trace shows the spectrum measured in this work. Expansion conditions are 25% water in He at 1.7-bar stagnation pressure and photoionization at 400 nm.

ice and liquid water, the maximum absorption is around  $3400\text{ cm}^{-1}$ . Thus, the transition between amorphous and crystalline ice should be revealed by the gradual shift to the lower lying peak in experiments with precisely size-selected clusters, as explained in detail by Buch *et al.* (6).

Electron diffraction measurements for water clusters give crystalline structures of cubic ice for the average cluster size  $\langle n \rangle = 5000$  (26, 27). A transmission electron microscopy experiment exhibits crystalline structures for even larger clusters in the range of  $\langle n \rangle = 0.5 \times 10^5$  to  $4.3 \times 10^5$  (2). In the experiment of Torchet *et al.*, the onset of the crystallinity is estimated to occur somewhere between  $n = 200$  and  $1000$  (26). A more precise determination could not be given because the exact information on the cluster size was not available.

In this work, IR spectra in the OH-stretching region ( $2800$  to  $3800\text{ cm}^{-1}$ ) were measured by using the IR-modulated photoionization scheme depicted in Fig. 1. Mechanistic aspects of the approach are elaborated below in detail. First, the clusters are formed in a supersonic expansion ( $353$  to  $403\text{ K}$  water temperature seeded with He at  $1.7$ - to  $4$ -bar stagnation pressure), skimmed, and then doped with a single Na atom in the pick-up cell. The residence time of the clusters in the pick-up zone is  $\sim 20\text{ }\mu\text{s}$ . After  $\sim 240\text{ }\mu\text{s}$  (depending on the beam velocity, which is typically  $\sim 1500\text{ m/s}$  for He-seeded expansions of water), the Na-bearing water clusters reach the zone for interaction with IR and UV-visible radiation. The excitation of the clusters with IR photons induces a signal enhancement to the UV photoionization when the UV pulse is delayed  $80$  to  $300\text{ ns}$ , depending on the focal parameters of the two laser beams (22, 24, 25). Further details are given in (19, 24) and the supplementary materials.

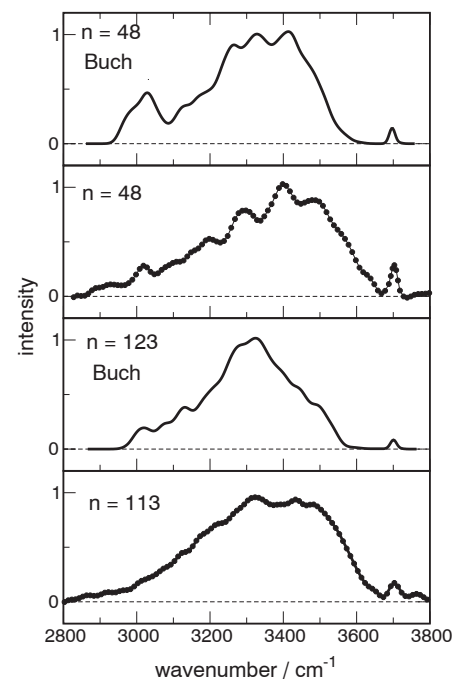
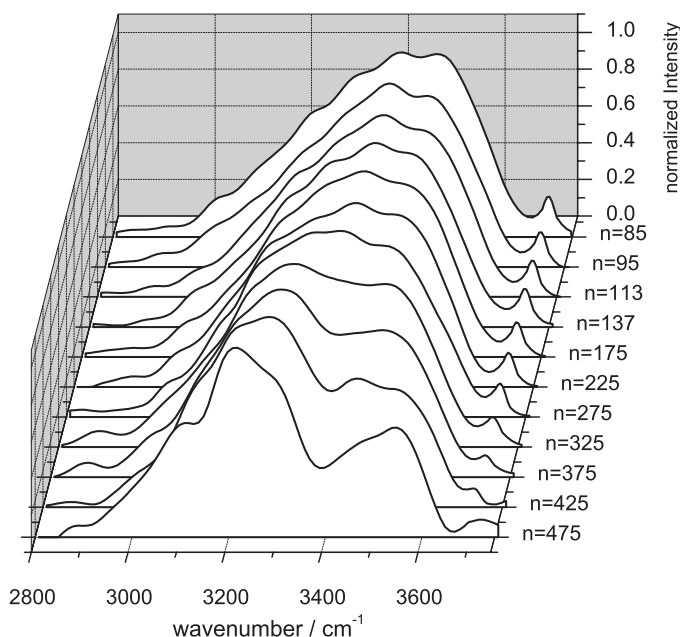
In two recent studies on small Na-doped methanol ( $n = 4, 5$ ) and water ( $n = 3$ ) clusters, we

have elaborated the action effect leading to signal enhancement. At expansion conditions favoring the production of cold clusters, the observed IR spectra were dominated by features of clusters with an exterior Na atom (24, 25), which means that the hydrogen-bonding network of the initially produced pure-solvent clusters is not changed upon Na attachment. The water trimer with exterior Na was kinetically stabilized during the Na pick-up process. In the case of cationic lithium-water clusters, the trapping of high-energy isomers was recently also reported (28). However, at the applied low-photon energies in our experiment, only clusters that have a partly solvated Na atom and a hydrated electron exhibit ionization potentials (IPs) low enough for photoionization. For signal saturation, the ionizing photon energies had to be increased by  $0.5$  to  $0.8\text{ eV}$ , indicating that the most abundant clusters have definitely higher IPs. Thus, the IR spectra showed that the IR photon energy is deposited in clusters with a nonionized Na atom situated in an exterior position, whereas the photoionization spectra strongly suggest that only clusters with an interior, partly solvated Na atom were photoionized. On the basis of the combined evidence from IR and photoionization spectra, we concluded that IR excitation induces a configuration change from isomers with an exterior Na atom to isomers with an interior Na atom. The configuration with an exterior Na, exhibiting a high IP, is predominant in the molecular beam, whereas isomers with an interior Na are much less abundant. These clusters show low IPs and define the appearance IP (which is the ionizing photon energy where the first ion signals are observed). At low photoionization energies, only clusters featuring interior Na atoms and solvated electrons are visible. These clusters contribute to the background signal. Upon IR excitation  $80\text{ ns}$  before photoionization, clusters with exterior Na

and high IP become visible through a configuration change of the heated cluster to a structure with lower IP (Fig. 1). This effect induces the exploited signal increase and is most probably enhanced by the higher absorption cross section of clusters featuring delocalized, solvated electrons. The mechanism is in agreement with molecular dynamics simulations in which we could demonstrate that a rise in cluster temperature—as the effect of IR excitation—dramatically increases the photoionization probability at the low-energy end of the photoionization spectrum (24).

In the present work, the Na atom is added to large water clusters. We showed in a recent study on the photoionization of this system that (i) the IPs of large Na-doped water clusters are size-independent between  $n = 15$  to  $450$  and that (ii) at photoionization energies of  $3.1$  to  $3.2\text{ eV}$ , only clusters that feature partly solvated Na atoms and the corresponding hydrated electrons are photoionized (29). Ion signal saturation is observed around  $4\text{ eV}$ , and raising the ionizing photon energies from  $3.3$  to  $4\text{ eV}$  increases the ion yields by more than a factor of  $6$ . This corresponds to the sharp rise of ion yields above  $3.3\text{ eV}$  ionizing photon energy depicted in Fig. 1, bottom. Clusters with an exterior, nonionized Na atom have IPs in the range of  $3.5$  to  $4\text{ eV}$ , which is  $0.7$  to  $1.2\text{ eV}$  above the appearance IP. This result was previously supported by theoretical investigations on Na-water and Na-methanol clusters (24, 25, 30) and is verified through exemplary calculations for larger Na-doped water clusters in the present work. These observations point to a high abundance of clusters with exterior, non-

**Fig. 2.** Evolution of size-selected  $(\text{H}_2\text{O})_n$  IR spectra from  $n = 85$  to  $475$ . 10 cluster sizes were averaged for  $n = 85$  and  $95$ , 25 cluster sizes were averaged for  $n = 113$  and  $137$ , and 50 cluster sizes were averaged for  $n = 175$  to  $475$ , which leads to significant noise reduction (text and supplementary materials). Expansion conditions are  $40\%$  water in He at  $(3.9 \pm 0.1)$ -bar stagnation pressure and photoionization at  $390\text{ nm}$ .



**Fig. 3.** Comparison of calculated IR spectra (6) for  $n = 48$  and  $123$ , with measurements at  $n = 48$  and  $113 \pm 12$ .



ionized Na atoms at the cluster surface, which is a similar situation to the case discussed above: Clusters with IPs well above the appearance IP dominate the molecular beam composition; at ionizing photon energies of 3.1 to 3.2 eV, only clusters with at least a partly solvated Na atom and rather low IPs are photoionized. If the IR excitation-induced signal increase is caused by a configuration change from structures with exterior to interior Na, the IR spectra should not be “contaminated” by features indicating Na solvation and solvated electron formation (22). Instead, the IR action spectra will resemble those acquired by photoionization of nonreactive chromophore molecules such as phenol. This is indeed the case, as is shown in Fig. 1, right, in which recently published IR spectra for phenol-(H<sub>2</sub>O)<sub>19</sub> are compared with Na(H<sub>2</sub>O)<sub>19</sub> (16). Taking into account the scatter of the experimental data, the spectra are equivalent. The phenol-(H<sub>2</sub>O)<sub>19</sub> spectrum was measured with photodissociation spectroscopy, demonstrating the great progress achieved with this technique by Fujii and co-workers. In the Na-water case, competing processes such as photodissociation are largely suppressed for expansions generating cold clusters [discussion in (24)]. In turn, inefficient photodissociation is probably a limitation of depletion techniques when applied to large clusters in molecular beam experiments. The production of large clusters leads in general to low-cluster temperatures, and large, cold clusters less efficiently dissociate upon IR excitation.

We will now briefly focus on the mechanism of the action effect for large clusters. Important aspects are the activation barriers involved and the temperature jump induced by IR excitation. Our previous work on singly doped Na-water clusters (29) suggested that the hydrated electrons observed in cluster studies are the key intermediates that are formed in the initial step of the Na-water ice surface reaction (29, 31, 32). The mechanism proposed in our work is quite general and was in subsequent work used to interpret solvated electron reactions at the vacuum-liquid interface (33). The Na ionization is the first step in a complex reaction scheme. For the next step—the charge transfer to a water molecule—Ferro

and Allouche reported a barrier of ~30 kJ/mol (34). The trapping of hydrated electrons on the time scale of cluster experiments (23) together with the observation by Kim *et al.* that all Na atoms are consumed after 3 s at 95 K (32) indicates that the barrier for the initial configuration change from a surface to a partly ionized Na atom is similar but rather below that of the electron transfer step—probably in the range of 15 to 25 kJ/mol. The barrier for the evaporation of a water molecule, which is a competing reaction path, is higher. For ice surfaces it is ~50 kJ/mol (35). This assessment of barriers is consistent with the proposed IR action effect: the enhanced formation of clusters with weakly bound hydrated electrons upon IR excitation.

The cluster temperature in our experiment and the induced temperature jump can be estimated from thermodynamic data reported by von Issendorff and co-workers for anionic water clusters (36). The melting point for anionic (H<sub>2</sub>O)<sub>118</sub><sup>−</sup> cluster is ~120 K. Because we observe solid clusters, this value gives an upper limit for cluster temperature in our experiment; the actual cluster temperatures are probably in the range of 90 to 115 K. Using a heat capacity of 0.25 meV/K molecule gives for  $n = 50$  a temperature jump of ~32 K when an IR photon of 0.4 eV (3250 cm<sup>−1</sup>) is absorbed. For  $n = 500$ , the increase is ~3 K; it is higher when more IR photons are absorbed (which is likely for large clusters). Temperature jumps in this order of magnitude should be large enough to accelerate the surface reaction. Regarding the ionization of the Na atom as a unimolecular isomerization reaction, the highly nonlinear reaction dynamics near the threshold of reactivity can drastically accelerate the reaction rate at moderately increased temperatures [for example, unimolecular decay rates of activated Na-acetic acid clusters in collision complexes (37)]. By changing the expansion and Na pick-up conditions as well as the ionizing photon energies, in cluster experiments the experimental focus also can be directed to different aspects of the Na-water reaction. Interesting issues in this context are the trapping of clusters that feature solvated electrons at lower cluster temperatures (22, 23). At higher pick-up cell temperatures, studies of reaction products are possible that provide complementary information to ice-surface studies with regard to the spectroscopic sensitivities as well as chemical and physical time scales (29, 31, 32, 38).

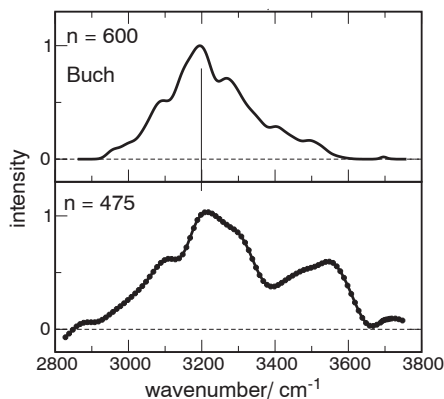
In the second part of this paper, the size-resolved infrared spectra are discussed. The evolution of OH stretch spectra with cluster size for the range  $n = 85$  to 475 is shown in Fig. 2. To increase signal intensities, the clusters are averaged over 10 to 50 cluster sizes, depending on the absolute cluster size (supplementary materials). Whereas the smaller clusters are dominated by peaks around 3400 cm<sup>−1</sup>, the main peak moves in the direction of 3200 cm<sup>−1</sup> for the larger ones. The transition occurs around  $n = 275$  and is already complete at  $n = 475$ . What we see in Fig. 2 is a typical transition from the solid amor-

phous behavior to a crystalline structure of the clusters. Such characteristics were exactly predicted by the pioneering calculations of Buch, which were based on an anharmonic oscillator model and the intermolecular coupling between the water molecules (6). Surface features as predicted by Buch and observed in collisional cooling cell experiments are also present.

The comparison of individual cluster sizes with calculated spectra is shown for  $n = 48$  and 123 in Fig. 3. The general trends, including the shift of the maximal intensity to smaller wavenumbers, are nicely reproduced. The features around 3000 and 3500 cm<sup>−1</sup> are traced back to undercoordinated surface molecules of dangling-H or dangling-O character, respectively. The latter features appear in the measurement with higher intensity, which may be related to the higher IR photon energy. As illustrated in fig. S1, the general shape of OH-stretch spectra of still amorphous clusters for sizes around  $n = 225$  is similar to those of cationic Na<sup>+</sup>(H<sub>2</sub>O)<sub>*n*</sub> clusters for  $n = 250$  (39). This observation confirms the conclusion of O'Brien and Williams that monovalent ions such as Na<sup>+</sup> do not affect the hydrogen-bonding network of the majority of water molecules in large clusters.

For the comparison of the crystalline cluster  $n = 475$ , we have chosen the lowest calculated size  $n = 600$  of this type in Fig. 4. The typical behavior with the peak around 3200 cm<sup>−1</sup> is present in both spectra and represents the four-coordinated crystalline structure. The shoulder at 3280 cm<sup>−1</sup>, which is also observed in the measurement, is according to the calculation an indication of four-coordinated molecules, with a distorted tetrahedral structure. The shoulders at 3400 to 3500 cm<sup>−1</sup> are indications of three-coordinated water molecules, signifying that the surface of these clusters is still amorphous. The comparison between the simulated spectrum for  $n = 600$  and the experimental spectrum for  $n = 475 \pm 25$  indicates that the crystallization sets in at smaller cluster sizes.

The general consistency of experiment and calculations allows us to use the predicted structures of Buch (6) to give the following, size-resolved picture of water crystallization in clusters: The first crystalline structure is in the center of the cluster and consists of a ring of six hydrogen-bonded water molecules in a tetrahedral configuration, with OH oscillators absorbing around 3200 cm<sup>−1</sup>. This motif is present for  $n = 275$ . Further addition of water molecules leads to a gradual growth of the crystalline core inside the cluster; at  $n = 475$  the spectral features of oscillators in the crystalline configuration dominate the spectrum, but surface and subsurface features are still present. The agreement of the calculations of Buch with the size-selective IR spectra has an important implication for larger clusters that were intractable for the applied theoretical method (6). Because the features observed in the spectrum for  $n = 475$  are also found in the OH stretch spectra of much larger clusters ( $\langle n \rangle = 1000$  to 100,000), it supports the



**Fig. 4.** Comparison of calculated (6) IR spectrum for  $n = 600$  with the measured one for  $n = 475 \pm 25$ .

proposed existence of subsurfaces in water nanocrystals (6). Torchet *et al.* gave a correct estimate for the transition between amorphous and crystalline behavior (somewhere between  $n = 200$  and 1000) based on the electron diffraction experiment (26).

The determination of the size range in which the critical nuclei of crystal water are formed is crucial information for resolving the crystallization mechanism of water. The onset of crystallinity defines a mechanistic branching point and affects barrier heights controlling water aggregation and hence measurable nucleation rates, visible morphologies, and macroscopic properties (40). In the future, it will be interesting to systematically study to what extent the crystallization process is a function of temperature, which can be influenced by the expansion conditions. The experimental technique presented here opens the door to systematic studies in this direction. Furthermore, the size-selected IR spectra in the OH-stretching region will provide benchmarks for theory to determine the predominate cluster structures in the size range of  $n = 50$  to 500. This information will help to refine water interaction potentials that are used for predicting macroscopic properties of water (40). More broadly, the method should be applicable to alcohols (24) and many other protic solvents, as well as other dopants (such as potassium and cesium).

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#### Supplementary Materials

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Supplementary Text  
Fig. S1

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## Structural Basis for Microtubule Binding and Release by Dynein

William B. Redwine,<sup>1,2\*</sup> Rogelio Hernández-López,<sup>1\*</sup> Sirui Zou,<sup>2</sup> Julie Huang,<sup>2</sup> Samara L. Reck-Peterson,<sup>2</sup> Andres E. Leschziner<sup>1†</sup>

Cytoplasmic dynein is a microtubule-based motor required for intracellular transport and cell division. Its movement involves coupling cycles of track binding and release with cycles of force-generating nucleotide hydrolysis. How this is accomplished given the ~25 nanometers separating dynein's track- and nucleotide-binding sites is not understood. Here, we present a subnanometer-resolution structure of dynein's microtubule-binding domain bound to microtubules by cryo-electron microscopy that was used to generate a pseudo-atomic model of the complex with molecular dynamics. We identified large rearrangements triggered by track binding and specific interactions, confirmed by mutagenesis and single-molecule motility assays, which tune dynein's affinity for microtubules. Our results provide a molecular model for how dynein's binding to microtubules is communicated to the rest of the motor.

**D**yneins are adenosine 5'-triphosphate (ATP)-driven molecular motors that move toward the minus ends of microtubules

(MTs) (1). The superfamily includes axonemal dyneins, which power the movements of cilia, and those that transport cargo, which include cytoplasmic dyneins 1 ("cytoplasmic") and 2 ("intraflagellar") (2). The transport of organelles, ribonucleoprotein complexes, and proteins by cytoplasmic dynein is required for cellular homeostasis, cell-cell communication, cell division, and cell migration (3), and defects in these processes result in neurological disease in humans

(4). Despite cytoplasmic dynein's role in such diverse activities and recent advances in characterizing its structure and motility, many aspects of its molecular mechanism remain poorly understood.

The core of the cytoplasmic dynein holoenzyme is a homodimer of ~500-kD motor-containing subunits (Fig. 1A). The major functional elements include (i) a "tail" domain required for dimerization and cargo binding (5); (ii) the force-generating "head" or "motor domain" (6, 7), a ring containing six AAA+ adenosine triphosphatase (ATPase) domains (8–10); (iii) a "linker" connecting the head and tail, required for motility (6, 7, 11, 12); (iv) the "stalk"—a long, antiparallel coiled-coil that emerges from AAA4 (13, 14); and (v) the MT-binding domain (MTBD), a small  $\alpha$ -helical domain at the end of the stalk responsible for binding the MT track (15–17). Unlike the other cytoskeletal molecular motors, kinesin and myosin, dynein does not have its ATPase and polymer track binding sites located within a single domain. With 25 nm separating AAA1, the main site of ATP hydrolysis, and the MTBD, an unresolved question is how dynein coordinates the cycles of nucleotide hydrolysis and MT binding and release required for its motion.

The mechanism coupling nucleotide hydrolysis to MT affinity has been suggested to be sliding in which the two helices in the stalk's coiled-coil

<sup>1</sup>Department of Molecular and Cellular Biology, Harvard University, 52 Oxford Street, Cambridge, MA 02138, USA. <sup>2</sup>Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA.

\*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: aleschziner@mcb.harvard.edu



adopt different registries by using alternative sets of hydrophobic heptad repeats (17–19). Dynein's stalk can be fixed in a specific registry by fusing it to a coiled-coil of known structure, such as that of seryl-tRNA synthetase (SRS) (18). Changing the length of the first stalk helix (CC1) relative to the second (CC2) shifts their alignment between high-affinity ( $\alpha$ ) and low-affinity ( $\beta$ ) registries and alters the affinity of the MTBD for MTs by up to a factor of 20 (Fig. 1B) (18). Engineered disulfide cross-linking in the monomeric dynein motor domain showed that its stalk explores multiple registries in solution and that a given registry is coupled to a specific MT affinity (19). Fixing the stalk registry also uncoupled the nucleotide state of the head from MT affinity (19).

Understanding the molecular mechanism by which stalk sliding is coupled to nucleotide hydrolysis and MT affinity would be aided by a structure of dynein's MTBD bound to MTs. Crystal structures are available for a low-affinity dynein MTBD fused to SRS through a short segment of the stalk (Fig. 1B) (17), and an adenosine

5'-diphosphate (ADP)-bound, presumably high-affinity dynein monomer (20). Only low-resolution structures of the MT-bound form have been reported (17, 21). Here we describe a cryo-electron microscopy (cryo-EM) reconstruction of the MTBD bound to MTs at subnanometer resolution, using a high-affinity version of the SRS-MTBD construct (18).

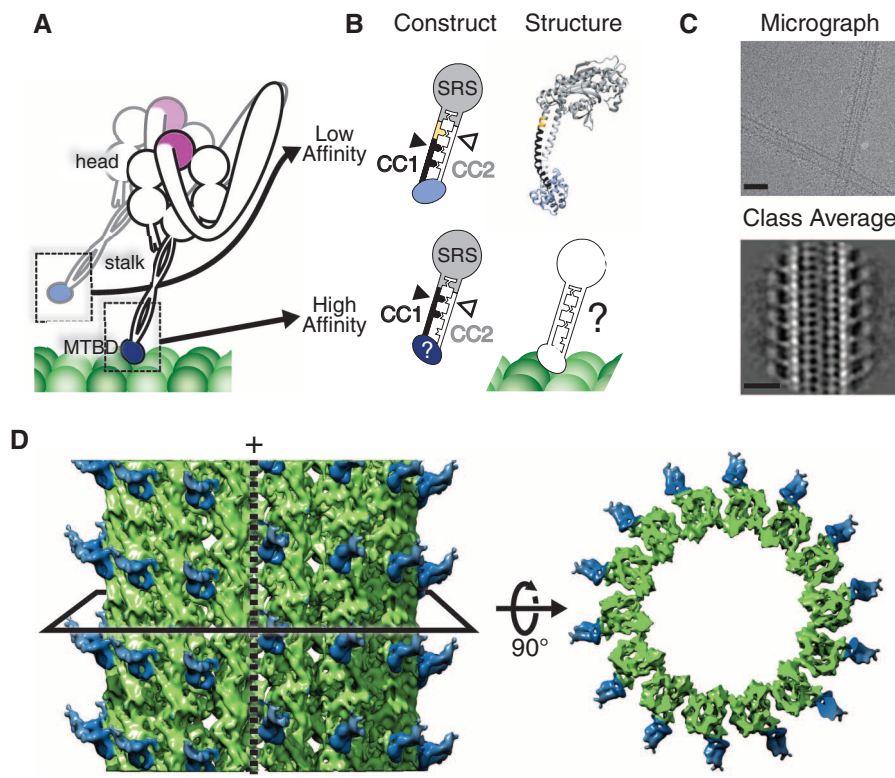
We collected images of MTs highly decorated with the SRS-MTBD under cryogenic conditions (Fig. 1C and fig. S1A) and adapted an image-processing method (22, 23) to solve its structure bound to 14-protofilament MTs. The SRS-MTBD binds to  $\alpha$ -tubulin and  $\beta$ -tubulin at the intradimer interface and is positioned to the side of the protofilament, as previously reported (17, 21) (Fig. 1D, fig. S1B, and movie S1). In the reconstruction, the portion including the MT, the MTBD, and the beginning of the stalk has a resolution of 9.7 Å (fig. S1D), where  $\alpha$  helices become visible.

We used molecular dynamics (MD) and our cryo-EM reconstruction to obtain pseudo-atomic models of the low- and high-affinity states of

the MTBD bound to MTs. First, we rigid-body docked the atomic-resolution structures of the tubulin dimer (24, 25) and the low-affinity MTBD into our map (Fig. 2, A and C; fig. S2A; and movies S1 and S2) and used MD to resolve steric clashes between a helix (H1) in the MTBD and the MT (Fig. 2, A and C; fig. S2, A and B; and movies S1 and S2). We then performed explicit-solvent molecular dynamics flexible fitting (MDFF) to shift the low-affinity MTBD structure to the high-affinity conformation in our reconstruction (fig. S2C). In addition to the MD force field, MDFF uses a potential-energy term derived from the experimental map and restraints on secondary structure to drive conformational changes that better fit the map (26, 27). The initial MDFF model agreed well within the experimental density of the MTBD (fig. S2C); however, the stalk remained in the low-affinity  $\beta$  registry present in the starting model. MDFF likely did not shift the registry of the coiled-coil due to the lower resolution at the tip of the stalk segment (fig. S1); a movement of the CC1 helix to the  $\alpha$  registry would make it protrude from the map, incurring a penalty in the simulations. We achieved the shift by applying targeted molecular dynamics (TMD) (28) to the tip of the coiled-coil (fig. S2D), using the  $C\alpha$  coordinates of the half-heptad shift in our construct to guide the final position of the stalk during the simulations. This final model (Fig. 2, B and D, and movie S3), which we refer to as the high-affinity MTBD, has the highest cross-correlation with the experimental map (figs. S2 and S3 and movies S4 and S5).

We repeated the MDFF calculations, using the MTBD from the recent crystal structure of an ADP-bound dynein monomer (20) (fig. S4). The only difference in the resulting pseudo-atomic model is in the stalk, where the dynein monomer's structure is missing density for one of the helices next to the MTBD (fig. S4, A and B). The similarity in the crystal structures of the MTBD in the low-affinity and ADP-bound states is likely due to the absence of MTs; our results suggest that the conformation we observe in our MT-bound high-affinity structure is stabilized by its interactions with  $\beta$ -tubulin (fig. S5A and tables S1 and S2). MDFF of the ADP-bound dynein monomer's MTBD into our cryo-EM map results in a large change in the angle between the MTBD and the stalk in the dynein monomer structure (fig. S4, A and B). This change makes the docking of the dynein monomer into our map compatible with previously reported measurements of the MT-stalk angle (17, 21) and our two-dimensional analysis of images of monomer-decorated MTs (fig. S4, D and E).

The cryo-EM map shows three points of continuous density between the MT and dynein's MTBD: the H1-H2 loop and helices H3 and H6 (Fig. 2B). Several parts of the structure are unchanged by its interaction with the MT, especially helices H6, H5, and CC2, with root mean square deviations (RMSDs) between those of the low- and high-affinity models of 1.4, 1.4, and 1.8 Å,



**Fig. 1.** Cryo-EM reconstruction of the cytoplasmic dynein high-affinity MTBD bound to a MT. (A) Schematic of dimeric cytoplasmic dynein. Major features relevant to this study are indicated. The MTBD is depicted in its low- (light blue) and high- (dark blue) affinity states during a step along the MT. (B) Schematic of the fusion constructs between the MTBD and seryl-tRNA synthetase (SRS) that fix the heptad registry of the stalk. The low-affinity construct has an additional four amino acids (yellow) inserted in CC1 (black) relative to the high-affinity construct. (C) Cryo-EM image of MTs highly decorated with the high-affinity SRS-MTBD construct (top) and a class average generated from segments of decorated 14-protofilament MTs (bottom). Scale bars: 50 nm (top) and 25 nm (bottom). (D) Three-dimensional reconstruction of the MTBD-MT complex, filtered to the calculated resolution of 9.7 Å (fig. S1). The black solid line represents a slice through the volume, which is shown on the right viewed from the minus end of the MT. The MT polarity is indicated and the dashed line shows the location of the MT seam. The SRS has been omitted due to its lower resolution (fig. S1).



respectively. The largest changes are the repositioning of H1 (RMSD = 10.1 Å) and an opening of CC1 in the coiled-coil next to the MTBD (RMSD = 8.1 Å) (Fig. 2, A to D, and movies S1, S2, S3, and S6), a movement anchored at the proline kink present in CC1. The final position of H1 is stabilized by multiple interactions with an acidic patch in H12 of  $\beta$ -tubulin not fully occupied in the low-affinity state (fig. S5A). This patch also stabilizes the high-affinity state of kinesin (29).

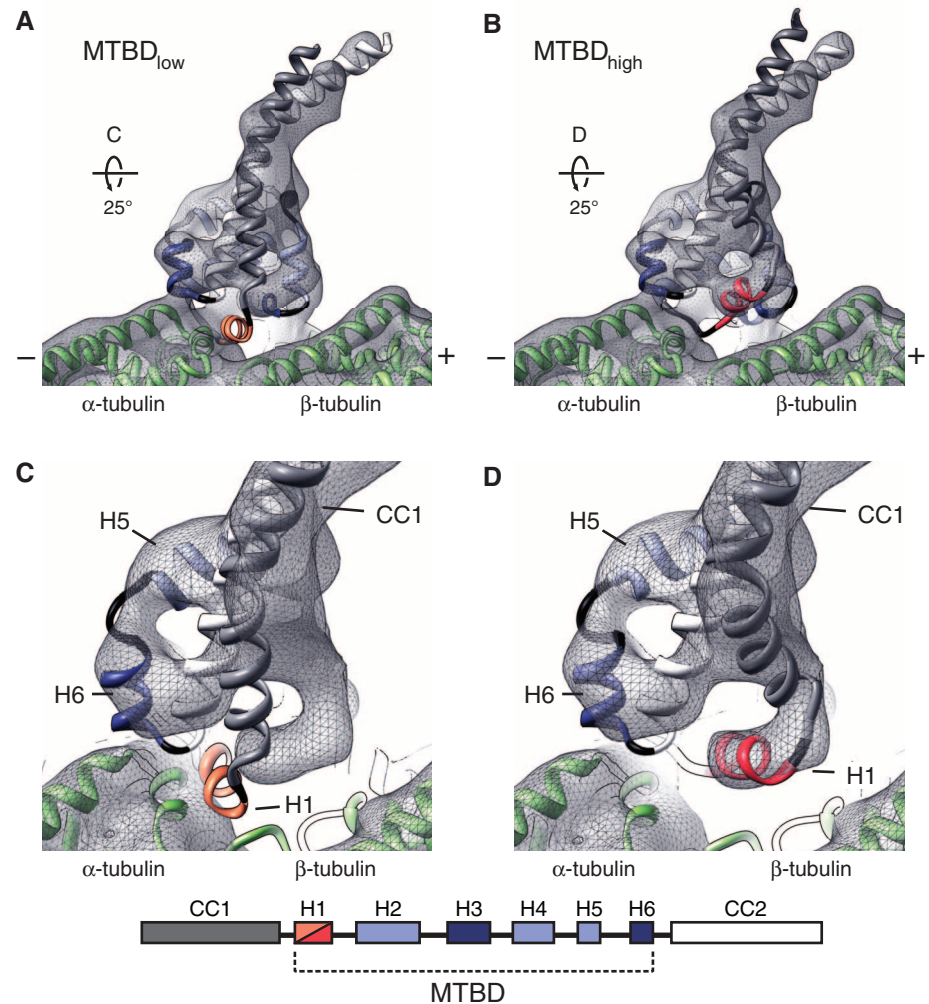
We monitored hydrogen bonds and salt bridges formed between the MTBD and the MT during MD simulations (tables S1 and S2); the high-affinity MTBD formed more hydrogen bonds with the MT (fig. S6) and electrostatic interactions at H1, H3, and H6 (fig. S5 and table S1). Nearly all of these residues are highly conserved, and mutating them results in defects in MT binding (18, 30) (fig. S5). The importance of salt bridges to the MTBD-MT interaction is consistent with the sensitivity of dynein's motility to ionic strength (fig. S7).

Our structural analysis suggested that the MTBD contains residues that lower its own affinity for the MT. In the MD simulations, basic residues in H1 and H6 formed salt bridges that alternated between intramolecular and intermolecular partners. In the MT-bound high-affinity conformation, H1-K3298 switched between a glutamate on  $\beta$ -tubulin and a conserved glutamate in CC1 (E3289) (Fig. 3A and fig. S8A); neither contact can be formed by H1-K3298 in the low-affinity conformation (Fig. S5A). H6-R3382 switched from an intramolecular interaction with a conserved glutamate in the same helix (H6-E3378) in the low-affinity unbound state to an intermolecular interaction with a cluster of glutamates on  $\alpha$ -tubulin upon binding (Fig. 3B and fig. S8B); the intramolecular interaction might weaken the MTBD-MT interaction in both the low- and high-affinity conformations. The importance of the two MTBD glutamates involved in the intramolecular salt bridges had previously been recognized; substitution of CC1-E3289 and H6-E3378 with alanine increased dynein's MT-binding affinity (18, 30) and reduced its ATP-stimulated release from MTs, respectively (30). We hypothesized that these phenotypes resulted from the competition between MT and MTBD residues in CC1 and H6 for salt-bridge formation with the basic residues K3298 and R3382 in the MTBD.

To test this prediction, we mutated the residues equivalent to E3289 and E3378 in CC1 and H6 of *Saccharomyces cerevisiae* dynein to either an isosteric but neutral (Q) or a basic amino acid (K) to disrupt the salt bridge (Q) or introduce an intramolecular charge repulsion (K) that may favor intermolecular interactions between H1-K3298 or H6-R3382 and acidic residues on the MT surface. Single-molecule motility assays that monitored the movement of purified mutant dyneins showed significant increases in dynein's run length and small decreases in velocity that

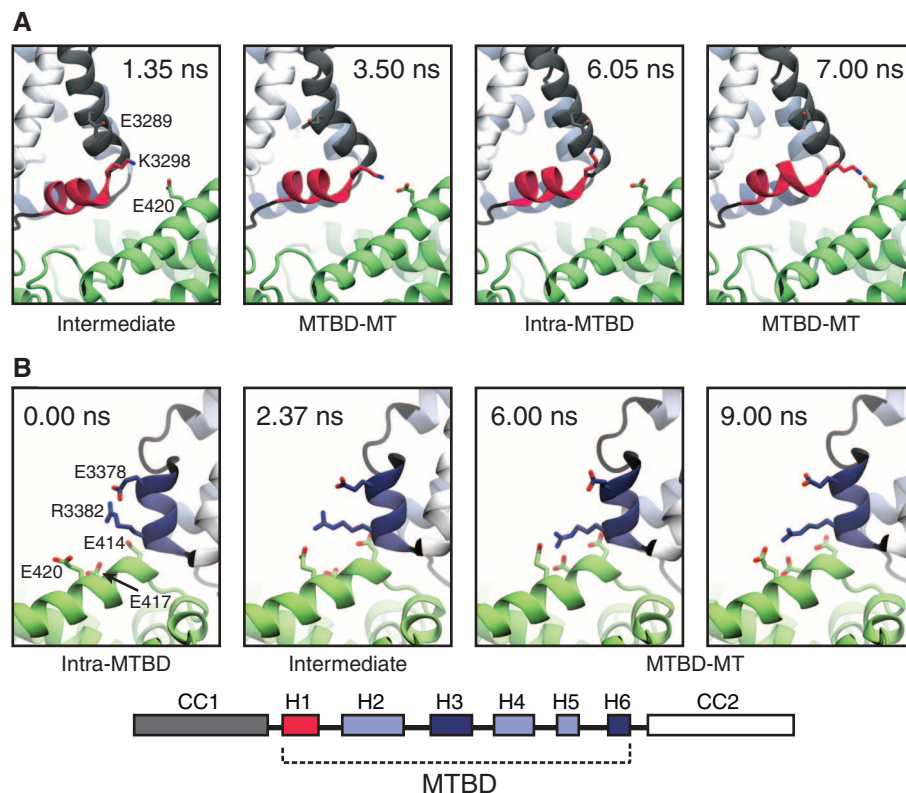
paralleled the severity of the mutation (E  $\rightarrow$  Q  $\rightarrow$  K) (Fig. 4, A and B, and fig. S9). Most notably, the basic substitutions CC1-E3289K and H6-E3378K increased dynein's run length by fivefold and sixfold, respectively (Fig. 4B), and the double mutant increased the run length even further (fig. S10). These results suggest that cytoplasmic dynein has been selected for submaximal processivity. The effects observed with the mutants are not due to a strengthened interaction with the unstructured C-terminal tails of tubulin (E-hooks). Although their removal decreased the run length of all constructs tested, in agreement with previous studies (31), the trend of increasing processivity (wild type  $\rightarrow$  E3289K  $\rightarrow$  E3378K) remained (fig. S11).

These findings provide a molecular model for how dynein couples its affinity for MTs with the nucleotide state of the motor domain (Fig. 4, C to E, and movie S6). We describe the transition from low to high affinity, but suggest that the proposed changes are reversible. During a diffusive search for its next binding site (Fig. 4C), an unbound MTBD is in the low-affinity conformation with its stalk in the  $\beta$ + registry, H1 oriented perpendicular to the MT axis and intramolecular salt bridges at key MT-binding residues. Consistent with this, a nuclear magnetic resonance study found that an unconstrained, minimal MTBD in solution exists in the  $\beta$ + registry and displays low affinity for MTs (32). Upon binding (Fig. 4D), transition to a high-affinity conforma-

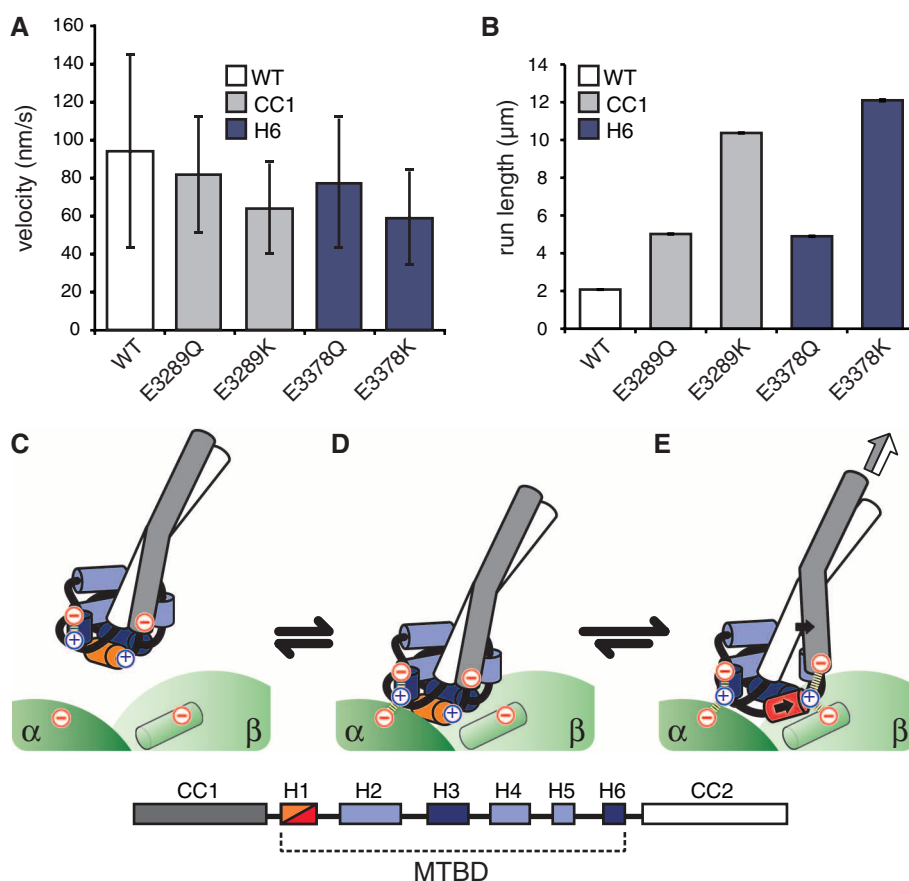


**Fig. 2.** The high-affinity, MT-bound state of the dynein MTBD is characterized by repositioning of helices H1 and CC1. (A) Rigid-body docking of the low-affinity MTBD structure into our cryo-EM density. (B) Pseudo-atomic model of the high-affinity MTBD bound to MTs generated by MDFF and TMD (see text for details). (C) Enlargement of the structure shown in (A), with its orientation indicated in (A). (D) Enlargement of the structure shown in (B), with its orientation indicated in (B). The cryo-EM map is shown as a transparent gray mesh. The MTBD is colored according to the scheme shown at the bottom of the figure, and structural elements are indicated in the different views. H1 (orange/red) is the element with the largest movement in the transition to the high-affinity conformation; H3 and H6 (dark blue) are major contact points with the MT (green).  $\alpha$ - and  $\beta$ -tubulin are indicated (green). MT polarity is indicated in (A) and (B). H1 protrudes from the cryo-EM map and clashes with the MT in the rigid-body docked low-affinity state (A and C).

**Fig. 3.** Behavior of dynamic salt bridges in the MTBD as determined by MD. **(A)** K3298 in H1 of the MTBD alternates between an intermolecular salt bridge with E420 on  $\beta$ -tubulin (MTBD-MT) and an intramolecular salt bridge with E3289 on CC1 of the MTBD (intra-MTBD). **(B)** R3382 in H6 of the MTBD alternates between an intermolecular salt bridge with E414 and E420 on  $\alpha$ -tubulin (MTBD-MT) and an intramolecular salt bridge with E3378 in H6 (intra-MTBD). Single-letter amino acid code and number are indicated for *Bos taurus* tubulin and *Mus musculus* cytoplasmic dynein (E, Glu; K, Lys; R, Arg). Time stamps for frames from MD simulations are indicated. "Intermediate" refers to a position midway between MTBD-MT and intra-MTBD salt bridges.



**Fig. 4.** Dynamic salt bridges reduce dynein motility. Bar graphs of **(A)** mean velocities and **(B)** characteristic run lengths of fluorescently labeled *S. cerevisiae* dynein bearing the equivalent of the indicated *M. musculus* mutations moving on MTs. Error bars: SD (A) and SE (B). Velocity and run length differences between wild type (WT) and Q (Gln) mutants, as well as between Q and K (Lys) mutations at the same position, are statistically significant (*t* test,  $P < 0.01$  for velocity, and two-tailed Kolmogorov-Smirnov test,  $P < 0.01$  for run length). The data for the double mutant (E→K at both CC1 and H6) was omitted because run lengths could only be determined under more stringent motility conditions (fig. S10). **(C to E)** Molecular model for the coordination of nucleotide state and MT binding by dynein (see text for details). (C) Unbound dynein in the low-affinity conformation; H1 is colored orange. (D) Initial interaction with a new binding site. (E) Repositioning of H1 (now in red) leads to the formation of new ionic interactions with  $\beta$ -tubulin (green cylinder) that stabilize the high-affinity state of the MTBD. The repositioning of H1 is accompanied by a movement in CC1; both movements are indicated by solid black arrows. The conformational change in the MTBD biases the registry of the coiled-coil toward the high-affinity  $\alpha$  state, a change that can propagate to the motor domain (white/gray arrow). Ionic interactions are indicated with dashed lines. The identities of the helices in the MTBD are indicated by the key.





tion involves a large displacement of H1, stabilized by new salt bridges with  $\beta$ -tubulin, and an opening of CC1 at the base of the stalk (Fig. 4E). The movements of H1 and CC1 likely constrain the registries that can be explored by the stalk, biasing the distribution toward the high-affinity  $\alpha$  registry (Fig. 4E). Propagation of this signal to the head would elicit conformational changes that produce a movement of the linker domain and a displacement of dynein toward the MT minus end.

Our analysis of dynamic salt bridges reveals that cytoplasmic dynein has been selected for submaximal processivity. Whereas kinesin has diversified its functional repertoire through gene duplication and divergence (33), cytoplasmic dynein is expressed from a single locus and may have evolved suboptimal processivity to increase the dynamic range of its regulation. High processivity could also be detrimental when multiple dyneins and kinesins must balance their actions on a single cargo (34). Consistent with this idea, intraflagellar dyneins, responsible for long, unidirectional transport within cilia (35, 36), contain neutral or basic residues at the equivalent of H6-E3378 (fig. S12), which would likely increase their processivity.

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#### Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6101/1532/DC1  
Materials and Methods  
Figs. S1 to S12  
Tables S1 to S3  
References (37–53)  
Movies S1 to S6

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## Specifying and Sustaining Pigmentation Patterns in Domestic and Wild Cats

Christopher B. Kaelin,<sup>1,2\*</sup> Xiao Xu,<sup>3,4\*</sup> Lewis Z. Hong,<sup>2</sup> Victor A. David,<sup>3</sup> Kelly A. McGowan,<sup>2</sup> Anne Schmidt-Küntzel,<sup>3,5</sup> Melody E. Roelke,<sup>3,6</sup> Javier Pino,<sup>7</sup> Joan Pontius,<sup>3,6</sup> Gregory M. Cooper,<sup>1</sup> Hermogenes Manuel,<sup>2</sup> William F. Swanson,<sup>8</sup> Laurie Marker,<sup>5</sup> Cindy K. Harper,<sup>9</sup> Ann van Dyk,<sup>10</sup> Bisong Yue,<sup>4</sup> James C. Mullikin,<sup>11</sup> Wesley C. Warren,<sup>12</sup> Eduardo Eizirik,<sup>13,14</sup> Lidia Kos,<sup>7</sup> Stephen J. O'Brien,<sup>3††</sup> Gregory S. Barsh,<sup>1,2†</sup> Marilyn Menotti-Raymond<sup>3</sup>

Color markings among felid species display both a remarkable diversity and a common underlying periodicity. A similar range of patterns in domestic cats suggests a conserved mechanism whose appearance can be altered by selection. We identified the gene responsible for tabby pattern variation in domestic cats as *Transmembrane aminopeptidase Q (Taqppe)*, which encodes a membrane-bound metalloprotease. Analyzing 31 other felid species, we identified *Taqppe* as the cause of the rare king cheetah phenotype, in which spots coalesce into blotches and stripes. Histologic, genomic expression, and transgenic mouse studies indicate that paracrine expression of *Endothelin3 (Edn3)* coordinates localized color differences. We propose a two-stage model in which *Taqppe* helps to establish a periodic pre-pattern during skin development that is later implemented by differential expression of *Edn3*.

The molecular basis and evolutionary variation of periodic mammalian color patterns have been difficult to investigate from

genetic crosses of model organisms. Domestic cats (*Felis catus*) exhibit heritable variation of tabby markings—mackerel versus blotched—that pro-

vide an opportunity for such genetic analysis (1). Tabby markings are a composite of two features: (i) a light background component in which individual hairs have extensive light bands, and (ii) a superimposed darker component in which hairs have little or no banding. In mackerel cats, the dark component is organized into narrow vertical stripes with a constant and regular spacing, whereas in blotched cats, the dark component is expanded into a less organized structure with wide whorls (Fig. 1A). Periodic color patterns in other felids may represent the same process; for example, dark tabby markings in domestic cats may be homologous to black stripes or spots in tigers or cheetahs, respectively (2).

A logical explanation for tabby patterning involves the Agouti-melanocortin receptor system, in which Agouti protein, a paracrine signaling molecule released from dermal papillae, acts on overlying hair follicle melanocytes to inhibit the melanocortin 1 receptor (Mc1r), causing a switch from the production of black/brown eumelanin to red/yellow pheomelanin (3, 4). According to this hypothesis, dark tabby stripes are areas in which Agouti signaling is suppressed or surmounted during hair growth and regeneration. However, known components of the Agouti-melanocortin



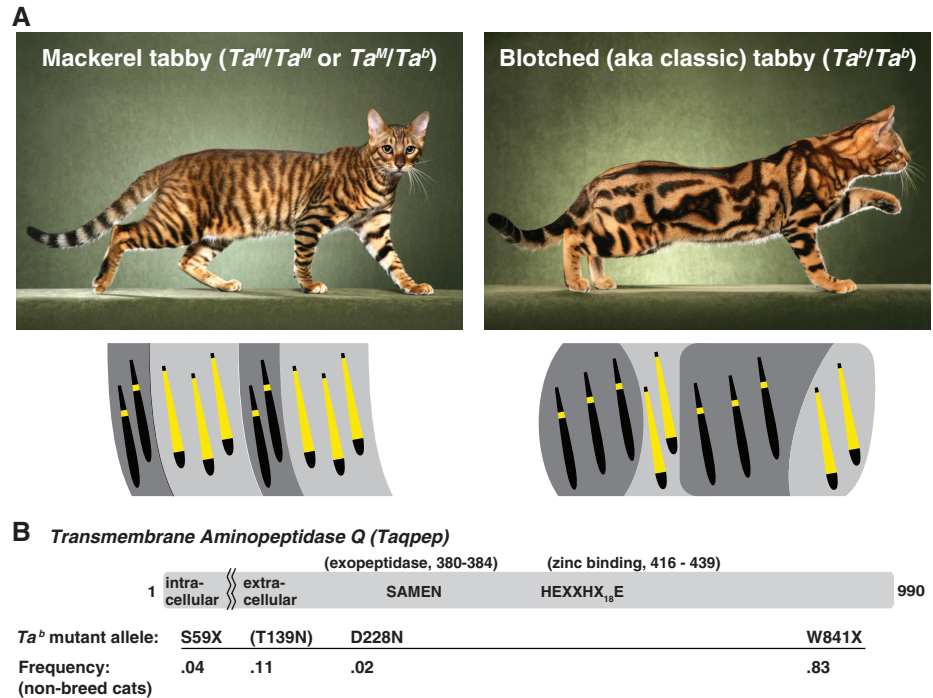
pathway do not affect the shape of tabby patterns (1, 2, 5, 6). Instead, the difference between mackerel and blotched is controlled by a single locus, *Tabby* (*Ta*), whose genetic position does not suggest an obvious candidate gene (7) but whose effects could be manifested via differential control of melanocortin signaling.

The 3X cat genome assembly is not contiguous across the *Tabby* linkage interval (7), but comparison to homologous regions in the dog and human genomes suggests a candidate interval of ~5 Mb in length (fig. S1A). Old World wild cats, from which domestic cats arose ~10,000 years ago (8), exhibit a mackerel-like pattern. However, the blotched pattern is common in many modern breeds, suggesting that one or a few *Ta<sup>b</sup>* causal variants would lie in a region of reduced allelic variation due to recent selection. We used comparative genomic information to identify single-nucleotide polymorphisms (SNPs) in the candidate interval, then genotyped and analyzed 16 blotched (*Ta<sup>b</sup>/Ta<sup>b</sup>*) and 33 mackerel (*Ta<sup>M</sup>/Ta<sup>M</sup>* or *Ta<sup>M</sup>/Ta<sup>b</sup>*) animals from a feral population in northern California. Five SNPs from a 180-kb interval on chrA1 showed significant association ( $P$  range =  $9 \times 10^{-4}$  to  $3.2 \times 10^{-9}$ ) (fig. S1B).

Twenty-four markers genotyped in and around the associated region in 58 blotched and 19 mackerel cats indicated that all blotched animals shared a common haplotype extending for 244 kb, whereas mackerel samples exhibited several haplotypes within the same interval (figs. S1C and S2). Coding sequences from three genes are located within the 244-kb interval: *Commd10*, *LOC644100*, and a third gene whose human ortholog has been referred to as both *Aminopeptidase Q* and *Laeverin* (9). No sequence alterations were observed in *LOC644100* or *Commd10*, but most blotched cats carried a nonsense mutation,

W841X (Fig. 1B, fig. S2, and table S1), in exon 17 of the third gene. We subsequently identified two additional variants in the same gene, S59X and

D228N (Fig. 1B, fig. S2, and table S1). This gene is expressed in developing felid skin, and its loss of function causes a loss of color pattern periodicity



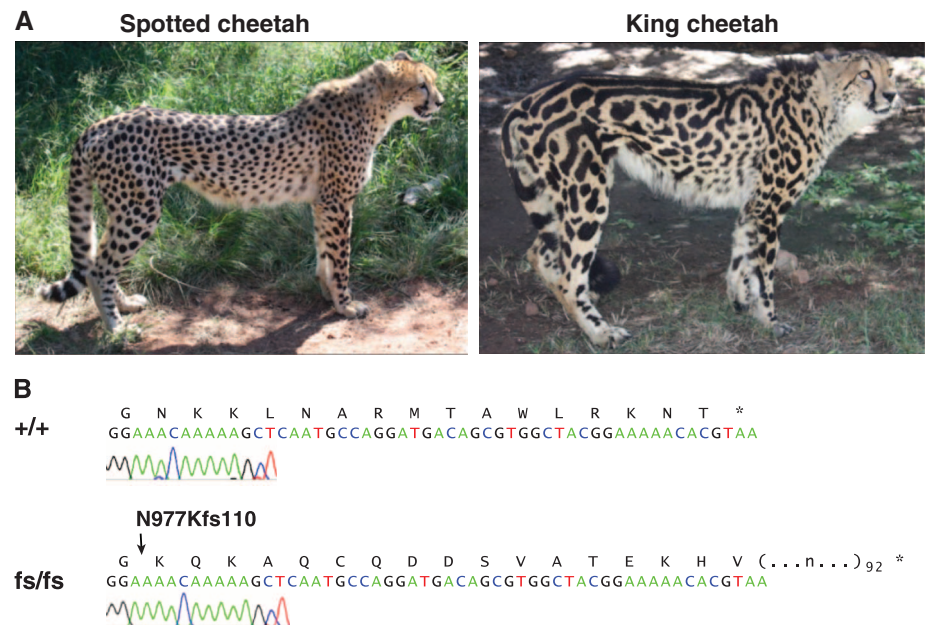
**Fig. 1.** (A) Allelic variation at *Tabby* [mackerel (*Ta<sup>M</sup>*) is dominant to blotched (*Ta<sup>b</sup>*)] controls the arrangement of dark- and light-colored areas. Diagrams indicate how the distribution of black or brown eumelanin versus yellow or pale pheomelanin within individual hairs underlies the macroscopic color patterns, although in reality cat hairs frequently exhibit multiple pheomelanin bands. (B) *Taqpep* encodes a type II membrane protein with aminopeptidase activity encoded by the ectodomain. Mutant allele frequencies are from a survey of 119 feral and outbred cats (table S1). The T139N allele is associated ( $P = 0.0017$ , Fisher's exact test) with an atypical swirled pattern but is incompletely penetrant (table S1 and fig. S2).

<sup>1</sup>HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA. <sup>2</sup>Department of Genetics, Stanford University, Stanford, CA, 94305, USA. <sup>3</sup>Laboratory of Genomic Diversity, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA. <sup>4</sup>Sichuan Key Laboratory of Conservation Biology on Endangered Wildlife, College of Life Sciences, Sichuan University, Sichuan 610064, China. <sup>5</sup>Cheetah Conservation Fund, Post Office Box 1755, Otjiwarongo, Namibia. <sup>6</sup>SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA. <sup>7</sup>Department of Biological Sciences, Florida International University, Miami, FL 33199, USA. <sup>8</sup>Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220, USA. <sup>9</sup>Veterinary Genetics Laboratory, Faculty of Veterinary Science Onderstepoort, University of Pretoria, Pretoria, South Africa. <sup>10</sup>The Ann van Dyk Cheetah Centre, De Wildt, South Africa. <sup>11</sup>Comparative Genomics Unit, National Human Genome Research Institute, National Institutes of Health, Rockville, MD 20892, USA. <sup>12</sup>The Genome Center, Washington University in St. Louis, St. Louis, MO 63108, USA. <sup>13</sup>Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul 90619-900, Brazil. <sup>14</sup>Instituto Pró-Carnívoros, Atibaia, Brazil.

\*These authors contributed equally to this work.

†To whom correspondence and requests for materials should be addressed. E-mail: gbarsh@hudsonalpha.org (G.S.B.); lgdchief@gmail.com (S.J.O.B.).

‡Present address: Theodosius Dobzhansky Center for Genome Informatics, St. Petersburg State University, St. Petersburg, Russia.



**Fig. 2.** (A) Black-haired areas are larger, more irregular, and associated with dorsal stripes in the king cheetah. (B) Chromatogram of *Taqpep* cDNA from a spotted (+/+) as compared to a king (fs/fs) cheetah.

without obvious effects on other organ systems. We refer to this gene as *Transmembrane Aminopeptidase Q* (*Taqpep*) and the protein product as Tabulin to reflect its organismal function.

*Taqpep* encodes a type II membrane-spanning protein of the M1 aminopeptidase family, whose members are characterized by the presence of GAMEN exopeptidase (SAMEN in *Taqpep*) and HEXHHX<sub>18</sub>E zinc-binding motifs in their extracellular domains (Fig. 1B) (9). In feral cats, we observed homozygosity or compound heterozygosity for the *Ta<sup>b</sup>* S59X or W841X alleles in 58 out of 58 (58/58) blotched animals, with no phenotypic distinction among the different genotypic classes, compared to 51/51 mackerel cats that carried 0 or 1 *Ta<sup>b</sup>* alleles (table S1). A third *Ta<sup>b</sup>* allele, D228N, was found to cosegregate with the blotched phenotype in a research colony (fig. S2D). In feral cats, we also observed two variants at codon 139, one of which, T139N, was significantly associated ( $P = 0.0017$ , Fisher's exact test) with an atypical swirled pattern (figs. S2 and S4D and table S1) and therefore may represent hypomorphic or neomorphic activity. Overall, the mutant W841X allele predominates and is responsible for the strong haplotype signature (fig. S1C), although the S59X allele occurs on the same haplotype background, in trans to W841X (fig. S2).

Cheetahs (*Acinonyx jubatus*) with the rare king pattern were originally described as a distinct felid species (10) but were later recognized as having a monogenic trait with an autosomal recessive mode of inheritance (11). In king cheetahs, the black spots coalesce into larger areas, and multiple longitudinal black stripes appear on the dorsum (Fig. 2A). Wild king cheetahs have been sighted only in a small area of sub-Saharan Africa (fig. S3) (11). *Taqpep* genomic sequence from a captive king cheetah in North America revealed a base pair insertion in exon 20, predicting a frameshift that replaces what would normally be the carboxy-terminal 16 amino acids with 109 new residues (N977Kfs110, Fig. 2B). Additional DNA samples from captive cheetahs in a large pedigree demonstrated complete cosegregation of the king pattern with the N977Kfs110 mutation [LOD (logarithm of odds) score = 5.7], and further revealed that the mutant allele was introduced into the pedigree by one homozygous and two heterozygous animals (fig. S3). We did not detect the N977Kfs110 mutation in wild cheetahs caught in Namibia ( $n = 191$ ), Tanzania ( $n = 23$ ), or Kenya ( $n = 3$ ).

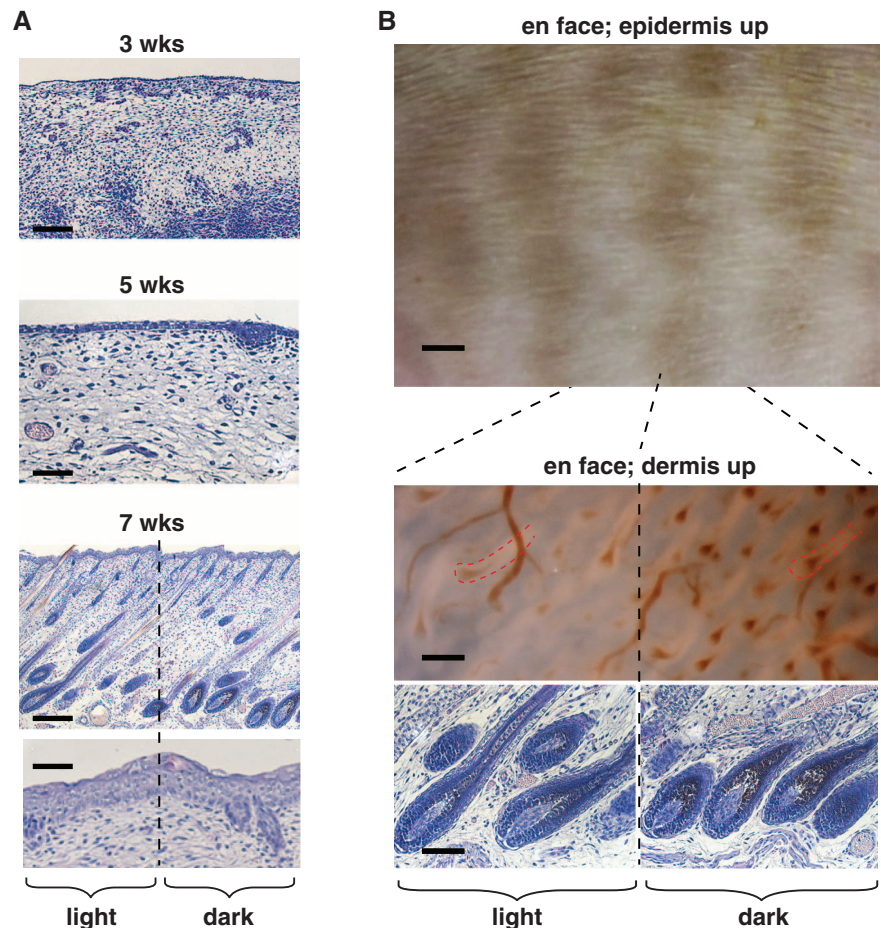
Depictions of tabby markings from the Middle Ages are mostly mackerel, but the blotched phenotype increased to a sufficiently high frequency to be described by Linnaeus in 1758 as characteristic for the domestic cat, predating the formation of most modern breeds. We examined the predicted Tabulin sequence in 351 cats from 24 breeds (table S2) and observed that the W841X allele is polymorphic in most breeds of Western origin, but rare or absent in Eastern breeds. The high allele frequency for W841X in Abyssinian (1.0), Birman

(0.71), and Himalayan (0.77) cats is especially notable, because tabby markings in these breeds are masked by epistatic interactions. The S59X allele, probably representing a complete ablation of protein function, is most common in Norwegian Forest Cats, and we observed one S59X/S59X homozygote with a blotched phenotype.

We determined *Taqpep* sequence for 31 wild felid species, identifying 130 synonymous and 64 nonsynonymous predicted substitutions (fig. S4A). We assessed the potential functional impact of the nonsynonymous substitutions with multivariate analysis of protein polymorphism (MAPP) (12), a quantitative approach that takes into account both evolutionary conservation and side-chain physicochemical properties. A MAPP score >10 indicates a likely impact on protein function, and the T139N and D228N substitutions associated with atypical swirled and blotched mutant phenotypes, respectively, in the domestic cat

yield MAPP scores of 11 ( $P = 1.5 \times 10^{-3}$ ) and 14 ( $P = 3.9 \times 10^{-4}$ ) (fig. S4B). In contrast, the T139A variant in the domestic cat (which does not affect pattern) yields a MAPP score of 3.8. The black-footed cat (*Felis nigripes*) is a clear outlier from other Felidae, with five lineage-specific nonsynonymous substitutions and a combined MAPP score of 50 (fig. S4C). *F. nigripes* exhibits a spotting phenotype that is similar to the atypical swirled pattern associated with the T139N allele in domestic cats (fig. S4D); thus, recent evolution of *Taqpep* in *F. nigripes* may contribute to its characteristic pattern.

To investigate how color patterns are implemented, we examined fetal cat skin at 3, 5, and 7 weeks of gestation, and observed that the first histologic indication of tabby markings coincides with their external appearance at 7 weeks, when follicle architecture is established and hair shafts begin to protrude through the epidermal surface



**Fig. 3.** Skin sections of fetal cats [(A), at 3, 5, and 7 weeks of gestation] stained with hematoxylin and eosin, together with unstained flat-mount (en face) skin preparations of fetal cats [(B), at 7 weeks of gestation]. The 7-week images in (B) are from an orange (O/Y or O/O) individual, which allows the dark component of the tabby pattern (which is orange-colored) to be more easily visualized. In the trans-illuminated “dermis-up” panel, hair follicle outlines (dashed red lines) appear light-colored; melanin incorporation and blood vessels appear dark-colored. Scale bars in (A), 150, 50, and 250  $\mu$ m in 3-, 5-, and 7-week fetal cat sections, respectively, and 50  $\mu$ m in the 7-week epidermis close-up. Scale bars in (B), 2 mm, 100  $\mu$ m, and 600  $\mu$ m in epidermis-up, follicle histology, and dermis-up panels, respectively.



(Fig. 3). At this stage, the boundary between dark and light tabby components reflects differences in the amount of melanin deposition, with no apparent difference in cell type; melanocytes are present in both dark and light areas but produce more melanin in dark areas. Furthermore, the density and architecture of hair follicles are independent of localization to dark and light areas (Fig. 3B). This suggests that tabby markings arise from spatial variation in transcriptional activity rather than cell type distribution.

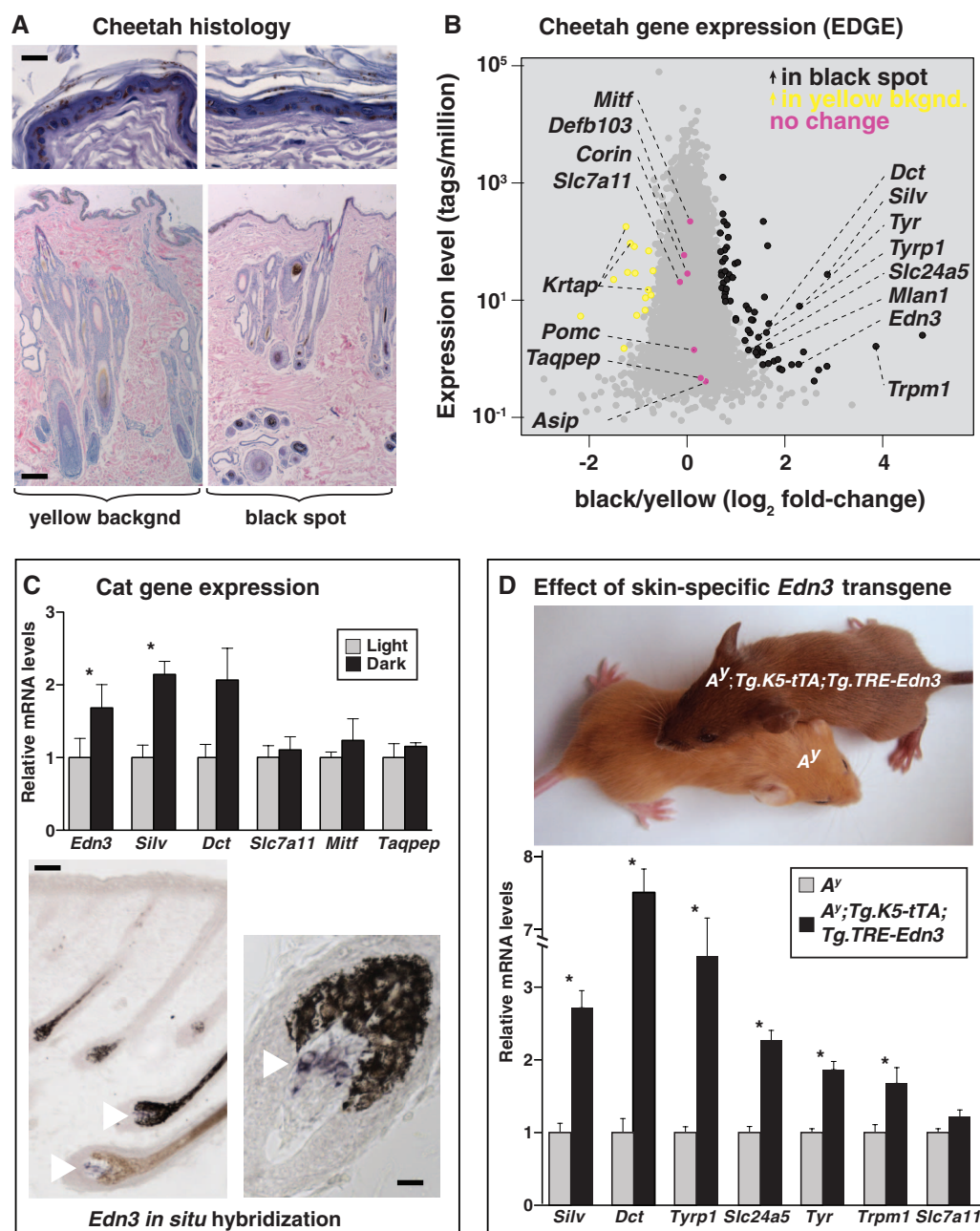
We used the EDGE (EcoP15I-tagged Digital Gene Expression) (13) method for gene expression profiling to examine the transcriptome of cheetah skin, in which there are sharp boundaries between black spots and the yellow interspot areas and for which multiple skin biopsies were

available for study. Cheetah skin exhibits an unusual histologic architecture; the epidermis is heavily pigmented, and hair follicles are organized in clusters with extensive accessory structures (Fig. 4A and fig. S5A). However, the number and size of follicle clusters, as well as the number of epidermal pigment cells, do not vary between black-haired and yellow-haired areas (fig. S5B). Based on a previous analysis of EDGE results from a single cheetah, we hypothesized that black coloration involves localized alterations of genes downstream of Mc1r signaling (13). Biopsies of yellow- and black-colored areas from five different animals enabled a genome-wide approach, in which the expression of 14,014 genes could be measured across a  $10^6$ -fold dynamic range (Fig. 4B). At a false discovery rate ( $q$ ) < 0.05, we

identified 74 differentially expressed genes (tables S7 and S8).

Among 60 genes up-regulated in black as compared to yellow cheetah skin, 7 are melanocyte-specific, of which 4 [*Tyr* (4.9-fold,  $q = 3.8 \times 10^{-19}$ ), *Silv* (7.3-fold,  $q = 1 \times 10^{-36}$ ), *Dct* (3.2-fold,  $q = 5.3 \times 10^{-8}$ ), and *Tyrp1* (3.1-fold,  $q = 1.7 \times 10^{-5}$ )] encode pigment type-switching components (14, 15). Genes that encode ligands or regulators of Mc1r signaling are not differentially expressed between yellow and black cheetah skin (Fig. 4B and table S7). However, one of the nonmelanocytic genes up-regulated in black as compared to yellow cheetah skin (4.9-fold,  $q = 2.1 \times 10^{-4}$ ), *Edn3*, a paracrine hormone expressed mostly by mesenchymal cells that promotes differentiation and proliferation of melanocytes and other neural

**Fig. 4. (A)** A cheetah skin biopsy that includes a black-yellow boundary (also see fig. S6). **(B)** EDGE (13) determination of differential gene expression in black- as compared to yellow-colored areas of cheetah skin. Transcript frequency (observations per million sequence reads, mean of five samples) is plotted as a function of differential expression. The 74 genes with significant differential expression are shown in black or yellow (FDR < 0.05, see methods and tables S8 and S9); 7 additional pigmentation genes that are not differentially expressed are shown in pink. **(C)** Relative mRNA levels (mean  $\pm$  SE) for the indicated genes as assessed by qRT-PCR from paired samples ( $n = 4$ ) of dark and light neonatal tabby skin;  $*P < 0.05$  (dark versus light, two-tailed  $t$  test). Dermal papilla expression of *Edn3* mRNA (purple stain, white arrows) as detected by in situ hybridization, from a brown tabby individual. The left panel illustrates the expression of *Edn3* mRNA in both pheomelanin- and eumelanin-containing follicles. **(D)** Phenotypes of 2-week-old mice of the indicated genotypes. Relative mRNA levels (mean  $\pm$  SE) for the indicated genes from qRT-PCR of cDNA from control ( $n = 4$ ) and transgenic ( $n = 4$ ) mice are shown;  $*P < 0.05$  ( $A^Y$  versus  $A^Y$ ; *Tg.K5-tTA*; *Tg.TRE-Edn3*, two-tailed  $t$  test). Scale bars in (A), 40 and 200  $\mu$ m in epidermis and whole-skin sections, respectively. In (C), 60 and 20  $\mu$ m left and right panels, respectively.





crest derivatives, is a candidate for the coordination of spatial variation in hair color. A hypermorphic mutation of *Gnaq*, the second messenger through which *Edn3* acts, causes the accumulation of dermal melanocytes during embryogenesis and converts yellow hair to black in postnatal mice (16, 17).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) confirmed that the expression of *Edn3* and *Silv* was increased in black-colored as compared to yellow-colored areas of cheetah skin; we also observed similar increases in a single leopard skin sample (fig. S5C). In neonatal skin from domestic cats (in which there is a high proportion of anagen follicles), *Edn3* mRNA expression was also ~twofold higher in dark as compared to light areas (Fig. 4C), and in situ hybridization revealed that expression was restricted to the dermal papilla, a permanent portion of the hair follicle.

In *A<sup>y</sup>* mutant mice, which express high levels of Agouti protein and produce pheomelanin instead of eumelanin, we confirmed that an *Edn3* transgene (18) converts yellow hair to dark brown-colored hair (Fig. 4D). Using qRT-PCR, we observed that the *Edn3* transgene in mice also caused increased expression of the same melanocyte-specific genes that are overexpressed in black-colored areas of cheetah skin (Fig. 4B). Thus, increased expression of *Edn3* in transgenic mice probably recapitulates both the coat color and

melanocytic gene expression phenotypes observed in cheetah skin, which suggests that localized expression of *Edn3* during felid hair follicle growth serves as a master regulator of spatial hair color differences associated with tabby markings.

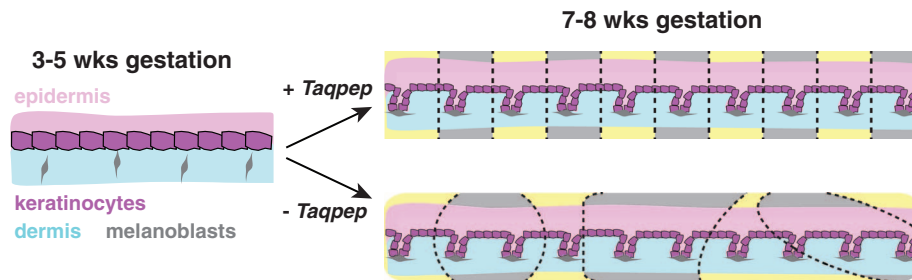
In the skin of neonatal domestic cats and adult cheetahs, *Taqpep* mRNA is expressed at low levels that do not differ between dark and light areas (Fig. 4, B and C). In mice, levels of whole-embryo *Taqpep* mRNA as measured by qRT-PCR increased progressively during gestation and were highest in postnatal skin (fig. S6). In situ hybridization to mouse embryos (at embryonic days 10.5 to 17.5) and to fetal cat skin at 3, 5, 6, and 7 weeks of gestation did not reveal localization of *Taqpep* mRNA to any specific cell type or region.

Because tabby markings are apparent soon after the time when melanocytes enter hair follicles (Fig. 3A), the effects of *Taqpep* on pattern morphology must occur at or before follicle development and are therefore likely to be mediated by epithelial or mesenchymal cells. Our results suggest that *Taqpep* is required to establish the periodicity of tabby markings during skin development (Fig. 5A), and that the “tabby marking” identity of a particular region is implemented and maintained by the ability of dermal papilla cells to sustain high versus low levels of *Edn3* production throughout subsequent hair cycles (Fig. 5B). This model also helps to explain why, in contrast to many

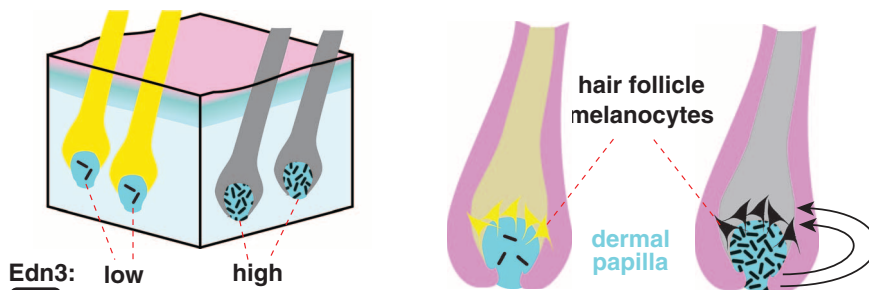
periodic color patterns in fish, which are mediated by and depend on direct contact between pigment cells (19–21), tabby markings change in size but not number during organismal growth.

Our findings also provide a mechanistic explanation for epistasis relationships between *Agouti*, *Mclr*, and *Tabby*. Homozygosity for a loss-of-function *Agouti* allele is associated with a “ghost pattern” in which Tabby stripes are difficult or impossible to visualize, because eumelanogenic genes such as *Tyr*, *Tyrp1*, *Dct*, and *Pmel* are already up-regulated. The ghost pattern becomes apparent, however, in animals that are doubly mutant for *Agouti* and *Mclr* (5), because endothelin and melanocortin signaling act in parallel (fig. S7). A system in which distinct paracrine signaling pathways—endothelins via a  $G\alpha_q$ -coupled receptor and melanocortins via a  $G\alpha_s$ -coupled receptor—converge on overlapping cellular machinery could explain more complicated phenotypes, in which pheomelanin-rich yellow versus eumelanin-rich black areas exhibit both periodic and regional fluctuation, such as spots or stripes of alternative pigment types overlaid on a background of dorsoventral differences, as is apparent in leopards, jaguars, and tigers. Further studies of color pattern in domestic-wild cat hybrids offer the opportunity to study these complex color markings and add to our knowledge of how felids acquire their color patterns.

## A Establishment of pre-pattern during fetal skin development



## B Implementation of pattern during follicle growth/regeneration



**Fig. 5.** A tabby pre-pattern is established at or before hair follicle development (A), specifying regions as dark (gray-colored) or light (yellow-colored). In the absence of *Taqpep*, dark regions are expanded, and there is less periodicity. Regional identity is manifested and implemented (B) by differential expression of *Edn3* in the dermal papilla, a permanent part of the hair follicle that releases paracrine factors to act on overlying melanocytes. Yellow and black pigment are synthesized by melanocytes in hair follicles that produce low and high levels of *Edn3*, respectively.

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#### Supplementary Materials

[www.sciencemag.org/cgi/content/full/337/6101/1536/DC1](http://www.sciencemag.org/cgi/content/full/337/6101/1536/DC1)  
Materials and Methods

Supplementary Text  
Tables S1 to S9  
Figs. S1 to S7  
References (22–36)

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# Loss of the Tumor Suppressor BAP1 Causes Myeloid Transformation

Anwesha Dey,<sup>1</sup> Dhaya Seshasayee,<sup>2</sup> Rajkumar Noubade,<sup>2</sup> Dorothy M. French,<sup>3</sup> Jinfeng Liu,<sup>4</sup> Mira S. Chaurushiya,<sup>1</sup> Donald S. Kirkpatrick,<sup>5</sup> Victoria C. Pham,<sup>5</sup> Jennie R. Lill,<sup>5</sup> Corey E. Bakalarski,<sup>4</sup> Jiansheng Wu,<sup>5</sup> Lilian Phu,<sup>5</sup> Paula Katavolos,<sup>6</sup> Lindsay M. LaFave,<sup>7</sup> Omar Abdel-Wahab,<sup>7</sup> Zora Modrusan,<sup>8</sup> Somasekar Seshagiri,<sup>8</sup> Ken Dong,<sup>9</sup> Zhonghua Lin,<sup>10</sup> Mercedesz Balazs,<sup>10</sup> Rowena Suriben,<sup>1</sup> Kim Newton,<sup>1</sup> Sarah Hymowitz,<sup>9</sup> Guillermo Garcia-Manero,<sup>11</sup> Flavius Martin,<sup>2</sup> Ross L. Levine,<sup>7</sup> Vishva M. Dixit<sup>1\*</sup>

De-ubiquitinating enzyme BAP1 is mutated in a hereditary cancer syndrome with increased risk of mesothelioma and uveal melanoma. Somatic *BAP1* mutations occur in various malignancies. We show that mouse *Bap1* gene deletion is lethal during embryogenesis, but systemic or hematopoietic-restricted deletion in adults recapitulates features of human myelodysplastic syndrome (MDS). Knockin mice expressing BAP1 with a 3xFlag tag revealed that BAP1 interacts with host cell factor-1 (HCF-1), O-linked *N*-acetylglucosamine transferase (OGT), and the polycomb group proteins ASXL1 and ASXL2 in vivo. OGT and HCF-1 levels were decreased by *Bap1* deletion, indicating a critical role for BAP1 in stabilizing these epigenetic regulators. Human *ASXL1* is mutated frequently in chronic myelomonocytic leukemia (CMML) so an ASXL/BAP1 complex may suppress CMML. A *BAP1* catalytic mutation found in a MDS patient implies that BAP1 loss of function has similar consequences in mice and humans.

Somatic inactivating *BAP1* mutations occur in the majority of metastatic uveal melanomas and approximately one-quarter of malignant pleural mesotheliomas. Somatic mutations also have been identified in breast, lung, and renal cell cancers (1–5). Recently, germline *BAP1* mutations were linked to a tumor predisposition syndrome characterized by melanocytic tumors, mesothelioma, and uveal melanoma (6, 7).

We investigated the normal physiological role of BAP1 using BAP1-deficient mice (fig. S1A). *Bap1*<sup>−/−</sup> embryos showed developmen-

tal retardation at embryonic day 8.5 (E8.5) and were not detected beyond E9.5, indicating that BAP1 is essential for embryo development (fig. S1, B and C). To bypass this embryonic lethality, we bred mice that expressed the tamoxifen-inducible recombinase creERT2 ubiquitously from the *Rosa26* locus (8) and had *Bap1* exons 4 and 5 flanked by lox sites (floxed) (fig. S1A). The floxed *Bap1* exons were deleted from most adult mouse tissues at 1 week after daily tamoxifen injections for 5 days were completed, the brain being the expected exception (fig. S1D). Loss of *Bap1* mRNA from hematopoietic lineages at 1 week after the final tamoxifen injection was confirmed by quantitative reverse transcription polymerase chain reaction (fig. S1E), and BAP1 protein was no longer detected in splenocytes by Western blotting (fig. S1F). Within 4 weeks of the last tamoxifen injection, 100% of the *Bap1*<sup>fl/fl</sup> creERT2<sup>+</sup> mice [hereafter referred to as BAP1 knockout (BAP1 KO) mice] developed splenomegaly (*n* = 12 mice). This phenotype was never observed in *Bap1*<sup>+/+</sup> creERT2<sup>+</sup> control mice [hereafter referred to as wild-type (WT) mice] (Fig. 1, A and B). Histopathology, flow cytometry, and myeloperoxidase immunohistochemistry revealed that splenomegaly in the KO mice resulted from extramedullary hematopoiesis and expansion of the myeloid lineage (Fig. 1, C to E). Myeloid cells also were increased in lymph nodes (fig. S2) and bone marrow (Fig. 1F).

Peripheral blood taken from 11 out of 12 BAP1 KO mice at 4 weeks after the final tamoxifen injection showed cytological features consistent with myelodysplasia and ineffective erythropoiesis (Fig. 1G). Total leukocyte numbers were elevated (Fig. 1H) because of monocytosis (Fig. 1I) and neutrophilia (Fig. 1J), which is consistent with chronic myelomonocytic leukemia (CMML)-like disease [classified as a myelodysplastic/myeloproliferative disease according to the new World Health Organization classification of myeloid neoplasms (9)]. Thrombocytopenia was detected as early as 1 week after the final tamoxifen injection (Fig. 1K), and all diseased mice developed severe progressive anemia (Fig. 1L). We noted morphologic features of erythroid dysplasia, including increased numbers of nucleated red blood cells, anisopoikilocytosis, and prominent basophilic stippling (Fig. 1L). Hypersegmented neutrophils (Fig. 1J), bilobed granulocytes (Fig. 1I), giant platelets (Fig. 1K), hyposegmented neutrophils consistent with pseudo-Pelger-Huët anomaly, and atypical immature cells with myelomonocytic features were also observed. Mitotic figures and apoptotic cells (fig. S3) were consistent with human myelodysplastic syndrome (MDS) (10), whereas blasts were rare. Collectively, these data show that *Bap1* deletion produces a myeloproliferative/myelodysplastic disorder with features of human CMML. Consistent with what is seen in patients with end-organ damage from myeloid neoplasms, the BAP1 KO heart contained microthrombi with multifocal necrosis, neutrophilic inflammation, and infiltration of myeloblastic cells (fig. S4).

Given that chronic myeloid neoplasms originate in the phenotypic hematopoietic stem cell (HSC) compartment (11), we characterized the lineage-depleted hematopoietic progenitor cell population in the BAP1 KO mice. Lineage-negative Sca1<sup>−</sup> c-Kit<sup>+</sup> myeloid progenitor cells and HSC-enriched lineage-negative Sca1<sup>+</sup> c-Kit<sup>+</sup> (LSK) cells were increased in BAP1 KO spleen and bone marrow as early as 2 weeks after the final tamoxifen injection (fig. S5A). Given that BAP1 KO mice develop monocytosis and neutrophilia, BAP1 KO LSK cells harvested 1 month after tamoxifen treatment expressed higher levels of a subset of genes involved in myelopoiesis [fig. S5, B and C (12)]. In methylcellulose colony-forming assays, BAP1 KO LSK cells yielded fewer colonies than WT LSK cells (fig. S6, A and B). In addition, unlike cells from WT colonies, which could be replated after 10 days in culture to form new colonies, replated BAP1 KO cells did not produce well-formed colonies, and many exhibited cytoplasmic blebbing characteristic of apoptosis (fig. S6, C and D). These in vitro data suggest that BAP1

<sup>1</sup>Department of Physiological Chemistry, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>2</sup>Department of Immunology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>3</sup>Department of Pathology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>4</sup>Department of Bioinformatics and Computational Biology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>5</sup>Department of Protein Chemistry, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>6</sup>Department of Safety Assessment, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>7</sup>Human Oncology and Pathogenesis Program and Leukemia Service, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. <sup>8</sup>Department of Molecular Biology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>9</sup>Department of Structural Biology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>10</sup>Department of Translational Immunology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. <sup>11</sup>MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.

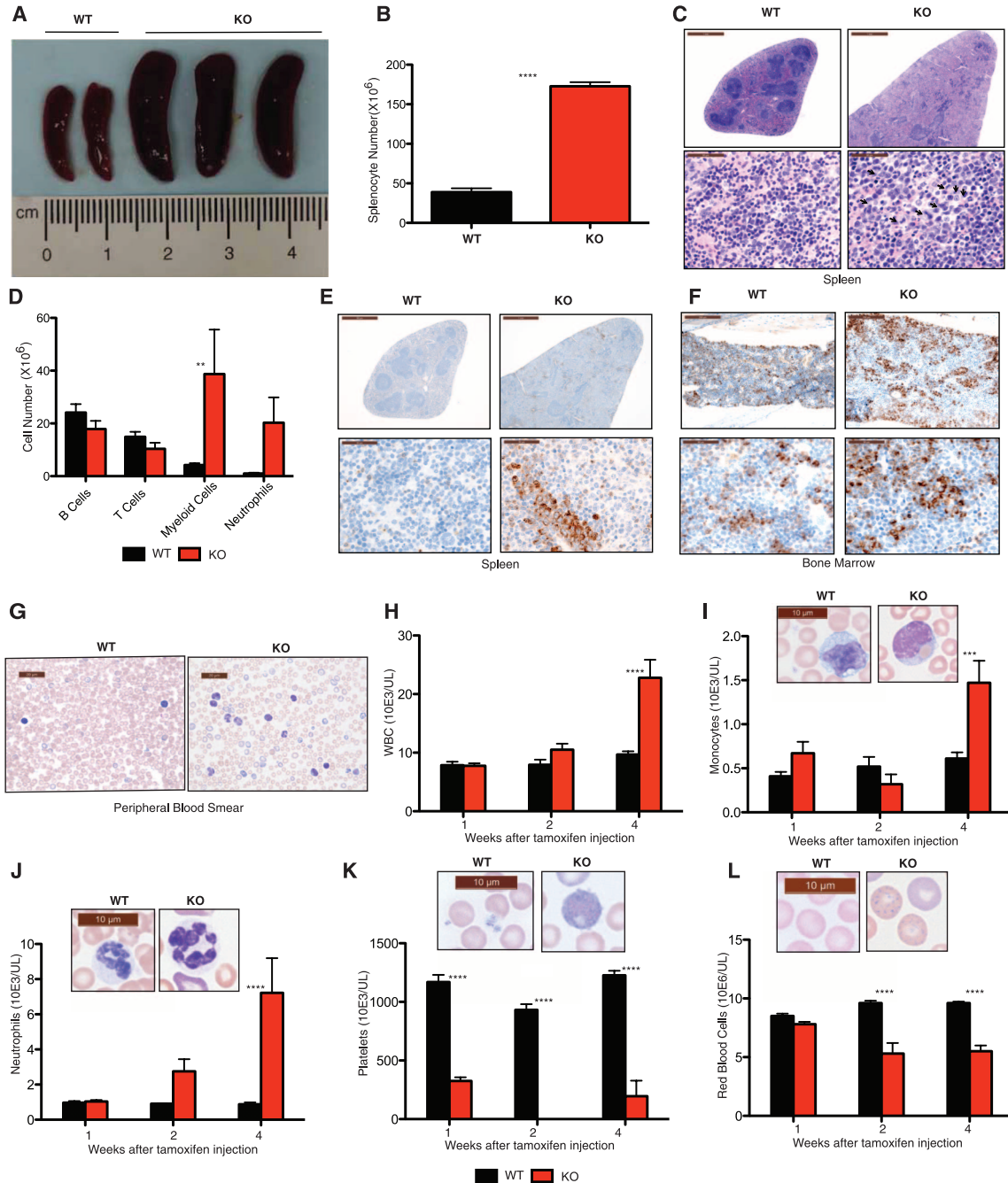
\*To whom correspondence should be addressed. E-mail: [dixit@gene.com](mailto:dixit@gene.com)

deficiency impairs HSC survival and/or self-renewal, but this may be context-dependent, in such a way that sufficient BAP1 KO HSCs survive in vivo to reveal the skewing of differentiation toward the myeloid lineage (Fig. 1 and fig. S5)

Next, we performed bone marrow transplantation studies to determine whether CMML-like disease is intrinsic to the BAP1 KO hematopoietic

compartment. BAP1 KO CD45.2<sup>+</sup> lineage-negative bone marrow cells harvested either 1 week (fig. S7A) or 1 month (fig. S7B) after the final tamoxifen injection were unable to reconstitute the bone marrow of lethally irradiated congenic CD45.1<sup>+</sup> B6.SJL recipient mice like their WT counterparts. This finding was confirmed in competitive repopulation assays in which recipient mice received equal numbers of WT B6.SJL and BAP1 KO

C57BL/6 bone marrow cells (fig. S8). Failure of the BAP1 KO cells to engraft might reflect an inability to home to the appropriate stem cell niche. However, *Bap1* deletion after the bone marrow of B6.SJL recipient mice was reconstituted with *Bap1*<sup>fl/fl</sup> creERT2<sup>+</sup> bone marrow cells (Fig. 2A and fig. S9) produced features of CMML, including thrombocytopenia (Fig. 2B), neutrophilia (Fig. 2C), monocytosis (Fig. 2D), and anemia (Fig. 2,



**Fig. 1.** BAP1 deficiency results in MDS/CMML-like disease. (A) *Bap1*<sup>+/+</sup> creERT2<sup>+</sup> (WT) and *Bap1*<sup>fl/fl</sup> creERT2<sup>+</sup> (KO) spleens at 4 weeks after tamoxifen treatment. (B) Total splenocytes at 4 weeks. (C) Hematoxylin and eosin staining of spleens at 4 weeks. (D) Leukocyte subsets in spleen at 4 weeks analyzed by flow cytometry. Identifying surface markers were B220 (B cells), CD3ε (T cells), CD11b (myeloid cells), and CD11b plus Gr1 (neutrophils). (E and F) Myeloperoxidase

staining of spleen (E) and bone marrow (F) at 4 weeks. (G) Peripheral blood smears at 4 weeks. (H to L) Peripheral blood cell counts. All graphs show the mean ± SD for five mice of each genotype. Asterisks indicate statistically significant differences between WT and KO mice based on two-way analysis of variance (ANOVA), followed by Bonferroni post-test analysis. 10E3/ul, 1 × 10<sup>3</sup>/ul; WBC, white blood cells. \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001.



E and F) at 4 weeks. Myeloid cells were increased in the spleen (Fig. 2G), and lineage-negative  $\text{Sca1}^+ \text{c-Kit}^+$  myeloid progenitor cells and LSK cells were increased in the bone marrow (Fig. 2, H to J). These data indicate that BAP1 deficiency restricted to the hematopoietic compartment is sufficient for the development of myeloid leukemia, and there is no requirement for BAP1 deficiency in the bone marrow stroma.

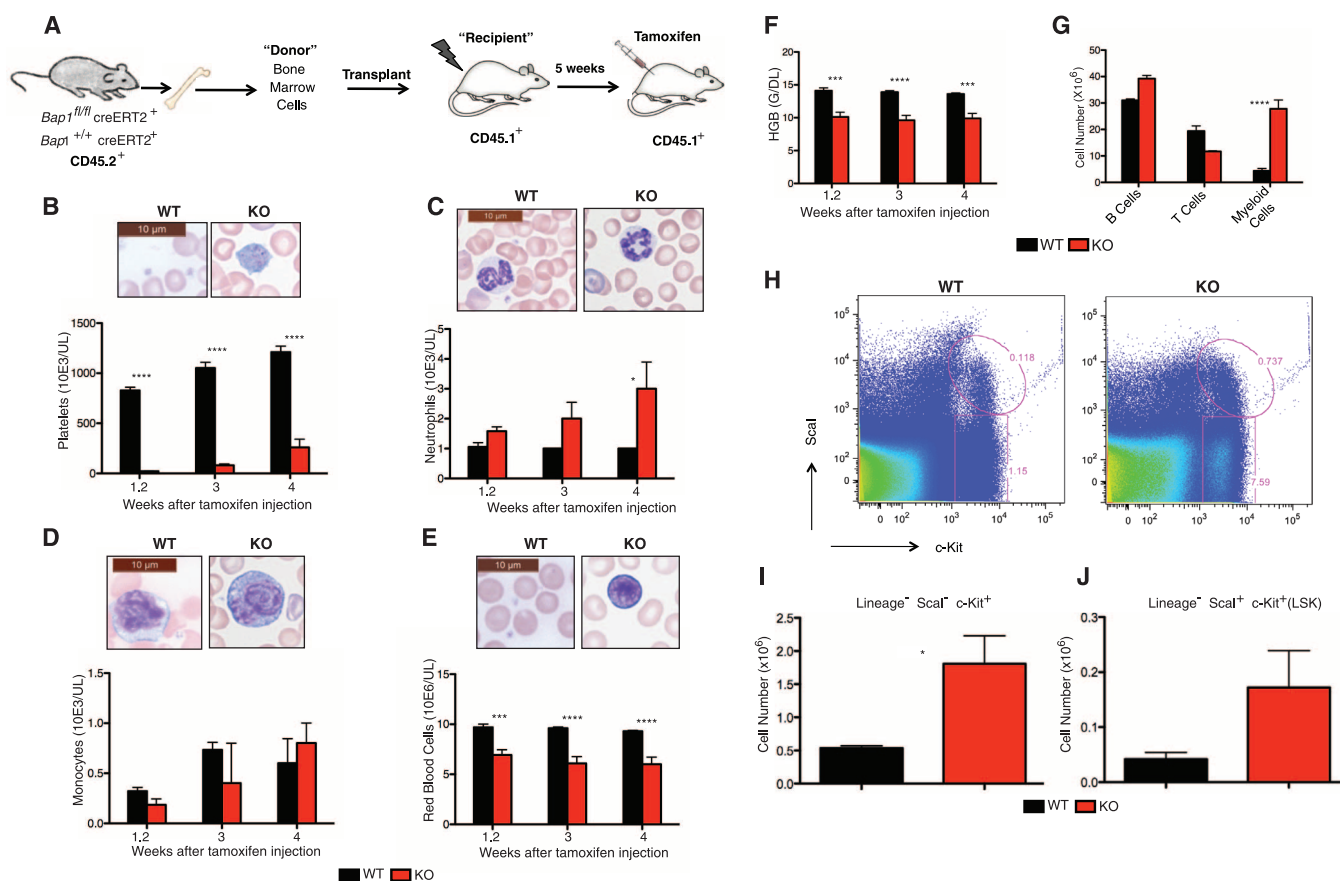
To investigate the mechanism for tumor suppression by BAP1, we characterized by mass spectrometry endogenous BAP1-interacting proteins, which we affinity-purified from BAP1.3xFlag knockin mouse spleen and brain (Fig. 3 and fig. S10A). These organs were chosen because they express BAP1 (fig. S10B), and their size means that protein yield is not a limiting factor. It is worth noting, however, that *Bap1* mRNA was detected in LSK cells (fig. S1E), and BAP1.3xFlag protein was immunoblotted in lineage-negative bone marrow cells containing the progenitor and stem cell populations (fig. S1I). Because antibodies to Flag also recognize several proteins in WT tissues (fig. S10B), we used a subtraction strategy

employing stable isotope labeling. Specifically, BAP1.3xFlag tissue lysates were mixed, before immunoprecipitation, in a 1:1 ratio with WT lysates prepared from animals fed  $^{13}\text{C}_6$ -lysine in their chow (13). If the light version of a peptide deriving from the BAP1.3xFlag knockin was not more abundant than its heavy WT counterpart when analyzed by mass spectrometry, then the protein was excluded as a BAP1-interacting protein. Immunoprecipitations with tissues from an unrelated knockin strain expressing 3xFlag.ARM8 (fig. S10C), which is a component of the CTLH (C-terminal to LisH motif) complex (14), provided a further control for specificity.

Captured proteins were washed briefly under low-stringency conditions, so that silver staining revealed no differential bands between WT versus BAP1.3xFlag spleen (fig. S10D). After the WT background was subtracted out, however, known BAP1-interacting proteins were revealed (15, 16), including host cell factor-1 (HCF-1), O-linked *N*-acetylglucosamine transferase (OGT), the polycomb group proteins ASXL1 and ASXL2 (Fig. 3A), the lysine demethylase KDM1B, and

the transcriptional regulator FOXK1. The BAP1 network defined by these experiments is represented in Fig. 3B, and it also includes select interactors from the STRING database or manually curated annotations if they connected to two or more proteins within our experimental data set. Immunoprecipitation and Western blotting of BAP1.3xFlag mouse spleen validated the interaction of OGT and HCF-1 with BAP1 (Fig. 3C), but we lacked quality antibodies to assess the other endogenous interactions in this manner. ASXL1 is a cofactor that is essential for BAP1 enzymatic activity (17) and is mutated frequently in MDS and CMML (18, 19). Given the development of MDS in our BAP1 KO mice, it is tempting to speculate that the BAP1-ASXL1 axis has a critical role in suppressing CMML.

De-ubiquitylation of the conserved epigenetic regulator HCF-1 by BAP1 prevents HCF-1 proteasomal degradation (20) and, consistent with this notion, BAP1 KO splenocytes contained far less HCF-1 than their WT counterparts (Fig. 3D). OGT also was decreased in BAP1 KO splenocytes (Fig. 3D), and, being the sole cellular enzyme



**Fig. 2.** BAP1 deficiency in hematopoietic cells is sufficient for MDS/CMML-like disease. (A) Bone marrow cells from  $\text{CD45.2}^+ \text{Bap1}^{+/+} \text{creERT2}^+$  or  $\text{Bap1}^{fl/fl} \text{creERT2}^+$  mice were transplanted into  $\text{CD45.1}^+$  lethally irradiated WT recipients. Tamoxifen was given to recipients at 5 weeks after transplantation to induce *Bap1* deletion. (B to F) Peripheral blood cell counts [(B) to (E)] and hemoglobin (HGB) levels (F) of reconstituted mice. (G) Splenic subsets at 4 weeks after tamoxifen treatment. Identifying surface markers were B220 (B cells), CD3 $\epsilon$  (T cells), and CD11b (myeloid cells). (H) Flow cytometric

analysis of lineage-negative bone marrow cell populations at 4 weeks after tamoxifen treatment. (I and J) Absolute numbers of lineage-negative  $\text{Sca1}^+ \text{c-Kit}^+$  myeloid progenitors (I) and lineage-negative  $\text{Sca1}^+ \text{c-Kit}^+$  (LSK) cells (J) in bone marrow at 4 weeks after tamoxifen treatment. All graphs show the mean  $\pm$  SD for three to five mice of each genotype. Asterisks indicate statistically significant differences between WT and KO mice based on two-way ANOVA, followed by Bonferroni post-test analysis. \* $P < 0.05$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

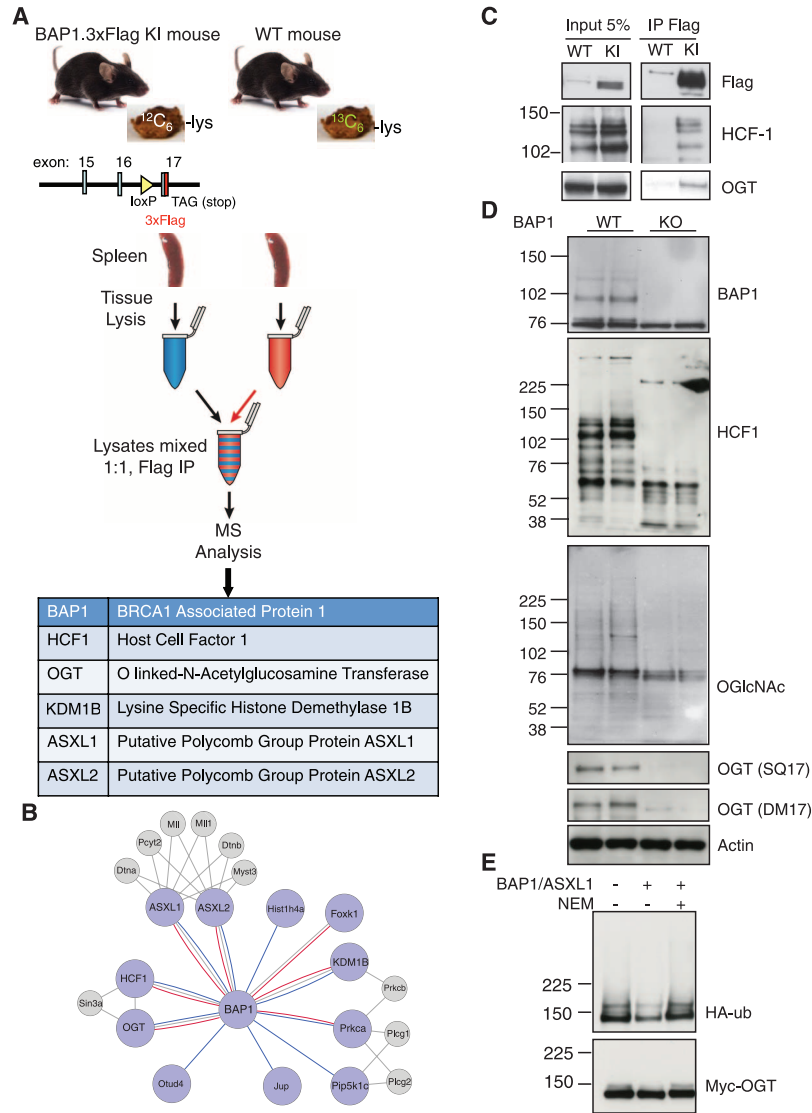
responsible for O-GlcNAcylation, the cells showed a corresponding decrease in O-GlcNAcylated proteins. To investigate whether BAP1 can deubiquitylate and stabilize OGT directly, rather than decreased OGT being secondary to a reduction in HCF-1, we affinity-purified ubiquitylated and Myc-tagged human OGT from HEK293T cells and then incubated it with human BAP1 and human ASXL1 residues 2 to 365, copurified from Sf9 cells. Consistent with OGT being a BAP1 substrate, ubiquitylation on OGT was reduced by the BAP1/ASXL1 complex, and this de-ubiquitylation was blocked when purified BAP1/ASXL1 was pretreated with the cysteine protease inhibitor *N*-ethylmaleimide (NEM) (Fig. 3E). NEM-mediated inactivation of BAP1 catalytic activity was confirmed in a ubiquitin-7-amido 4-methylcoumarin (Ub-AMC) cleavage assay (fig. S12A). In contrast to the situation in splenocytes, BAP1 deficiency in mouse embryo fibroblasts did not alter steady-state OGT protein abundance, but, in keeping with OGT being a BAP1 substrate, increased OGT protein turnover was revealed when translation was inhibited with cycloheximide (fig. S12B). Because O-GlcNAcylation of HCF-1 by OGT is necessary for the proteolytic maturation of HCF-1 (21, 22), our data indicate that BAP1 not only stabilizes HCF-1 but also is necessary for HCF-1 activation via OGT stabilization.

HCF-1 is a pleiotropic transcriptional coregulator (23), so BAP1 probably regulates gene expression via HCF-1 stabilization. To identify potentially critical BAP1-regulated genes, we determined what genes are (i) dysregulated in BAP1 KO cells by microarray analyses, and (ii) normally have BAP1 bound indirectly to their promoters (via yet-to-be-characterized DNA binding proteins), as judged by chromatin immunoprecipitation and DNA sequencing (ChIP-seq). Low chromatin yields from hematopoietic stem/progenitor cell populations precluded ChIP-seq studies, so we used BAP1.3xFlag knockin bone marrow-derived macrophages (BMDMs) as a surrogate to identify genome-wide BAP1 binding sites. A total of 9128 significant BAP1 peaks were identified after ChIP with antibodies to Flag (assessed with MACS analysis software with stringent criteria: *P* value < 1 × 10<sup>-10</sup> and fold enrichment >5). Of these sites, 5926 (65%) were located near the transcription start sites of 5731 genes (Fig. 4A). Motif enrichment analysis of the BAP1-binding sites revealed that the top two occurring motifs are most similar to known binding sites of the Ets (CGGAAG) family of transcription factors and of SP1 (GGGCGGGG) (Fig. 4B). Gene expression data sets were generated with WT and BAP1 KO lineage-negative Sca1<sup>+</sup> c-Kit<sup>+</sup> myeloid progenitor cells, as well as LSK cells. Among the 5731 genes with BAP1 peaks by ChIP-seq, 32 were down-regulated significantly in BAP1 KO cells (Fig. 4C), whereas 18 were up-regulated. We validated BAP1 localization to 30 out of 32 of the down-regulated genes by ChIP-qualitative polymerase chain reaction (qPCR) (Fig. 4D). Several of the genes identified affect the immune system, including *Il7r*, which is particu-

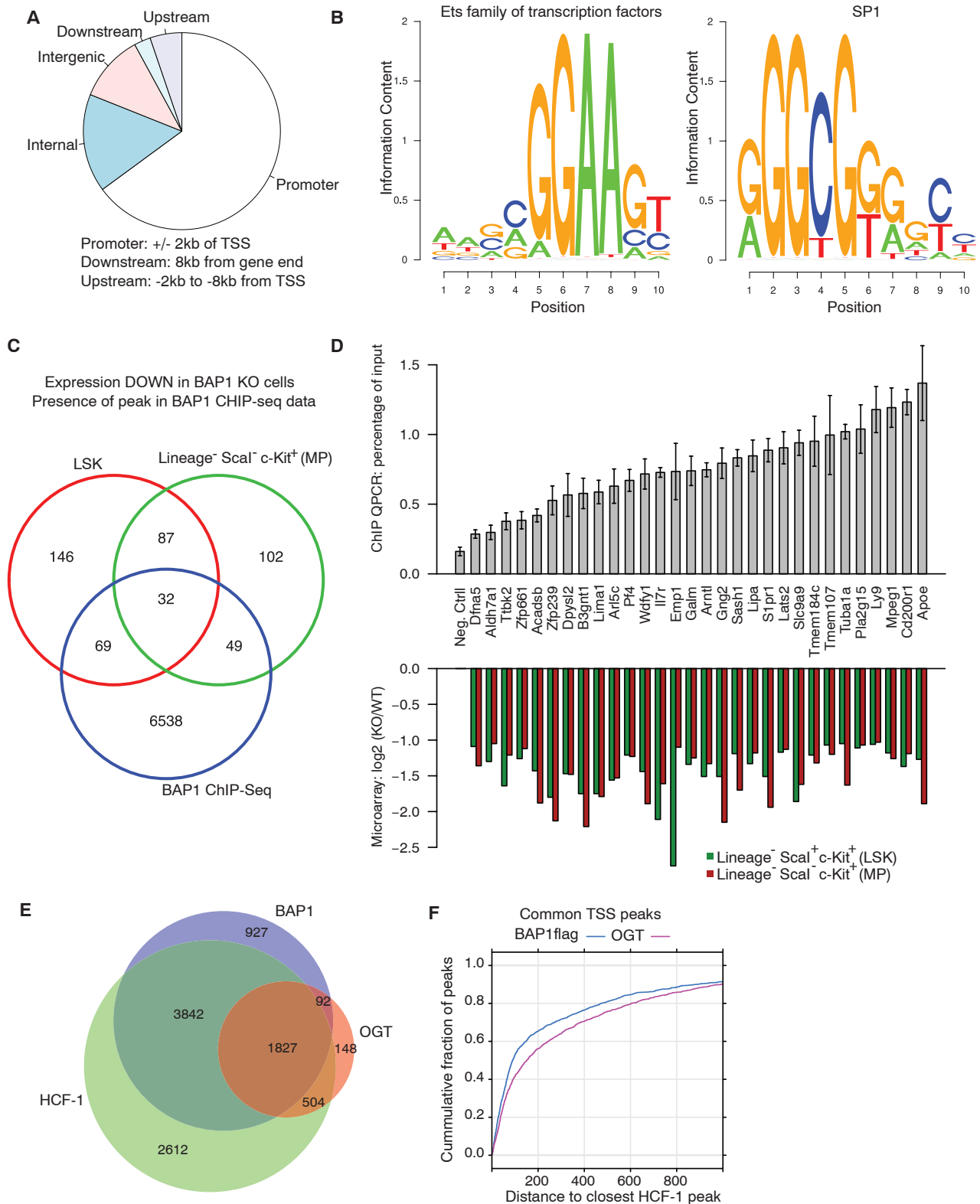
larly interesting as a candidate BAP1 target gene because it is a known regulator of hematopoietic cell survival, and *Il7R* expression is decreased in MDS patients (24).

Next we performed additional ChIP-seq studies for HCF-1 and OGT using WT BMDMs, the goal being to determine which promoters occupied by BAP1 also contain HCF1 and/or OGT. Most HCF-1 and OGT peaks (57.3 and 52.4%, respectively) were located near transcriptional start sites

(fig. S13). Eighty-five percent of promoters occupied by BAP1 also contained HCF-1 (Fig. 4E). Fewer OGT sites were identified, but of these, most were found in genes containing BAP1 and/or HCF-1 sites (Fig. 4E). Within the 1827 promoters occupied by BAP, OGT, and HCF-1, more than 65% of BAP1 peaks are within 200 base pairs (bp) of an HCF-1 peak, whereas 70% of OGT peaks are within 400 bp of an HCF1 peak (Fig. 4F). The close proximity of the BAP1, OGT, and HCF-1 peaks



**Fig. 3.** Identification and characterization of BAP1-associated proteins in mouse brain and spleen. (A) BAP1.3xFlag knockin (KI) mice fed regular chow and WT mice fed “heavy” <sup>13</sup>C<sub>6</sub>-lysine in their chow were used to make tissue lysates for anti-Flag immunoprecipitation (IP) and mass spectrometry (MS). Top IP hits that were represented by multiple unique peptides deriving primarily from the “light” BAP1.3xFlag KI are listed. (B) The mouse BAP1 interactome. Large nodes represent BAP1-interacting proteins identified in the brain (blue lines) or spleen (red lines). Gray lines indicate known interactions from the STRING database. Smaller gray nodes represent proteins from the STRING database that are connected to two or more proteins from our data set and include manually crated annotations. (C) Anti-Flag immunoprecipitations from WT and BAP1.3xFlag splenocytes. (D) Western blot analysis of WT and BAP1 KO splenocytes at 3 weeks after tamoxifen treatment. (E) Myc-tagged OGT was affinity-purified from HEK293T cells cotransfected with hemagglutinin (HA)-tagged ubiquitin and then incubated with BAP1/ASXL1 purified from Sf9 cells. Where indicated, BAP1 protease activity was inactivated with NEM before OGT was added.



**Fig. 4.** Identification of BAP1-regulated genes. **(A)** Characterization of BAP1 binding sites identified by anti-Flag ChIP-seq analysis of BAP1.3xFlag BMDMs. TSS, transcription start site. **(B)** De novo motif enrichment analysis of BAP1 binding sites identified similarities to Ets (CGGAAG) and SP1 (GGGCGGGG) transcription factor binding sites. **(C)** Venn diagram of genes with BAP1 recruited to their promoter in BAP1.3xFlag BMDMs (blue circle), and genes with reduced expression in BAP1 KO lineage-negative Scal<sup>-</sup> c-Kit<sup>+</sup> myeloid progenitors (MP; green circle) or BAP1 KO LSK cells (red circle). **(D)** Putative BAP1 target genes identified in (C) were val-

idated by ChIP-qPCR on BAP1.3xFlag BMDMs (top panel). Negative control (Neg. Ctrl) primers amplify a region in a gene desert, and therefore binding of transcription factors is not expected. Bars show the mean  $\pm$  SD of triplicate wells, the chromatin deriving from BMDMs pooled from multiple KI mice. The lower panel shows microarray expression data for these genes in WT versus BAP1 KO MP or LSK cells. **(E)** Venn diagram showing the overlap between promoters occupied by BAP1.3xFlag, HCF-1, or OGT, based on ChIP-seq analyses. **(F)** Distances separating BAP1 peaks and OGT peaks from HCF-1 peaks by ChIP-seq.



(Fig. 4F) fits with their being recruited to promoters as a complex.

Given that BAP1 KO mice develop MDS, we investigated whether *BAP1* mutations occur in human MDS by full-length resequencing of *BAP1* in 32 paired tumor and normal samples from patients with de novo MDS. We identified a patient with a frameshift mutation that causes premature termination within the UCH catalytic domain of BAP1. Analysis of matched normal DNA did not identify the frameshift allele, which is consistent with somatic acquisition of the frameshift *BAP1* mutation by the MDS clone (fig. S14). The patient with the somatic *BAP1* mutation presented with refractory cytopenias and multilineage dysplasia, similar to the multilineage dysplasia and cytopenias seen in our murine model. Mutational profiling revealed that this patient was WT for known MDS mutations, including *TET2*, *ASXL1*, *EZH2*, *NRAS*, *C-KIT*, *FLT3*, *IDH1*, and *IDH2*, and had del(20)(q11.2q13.3) as the sole cytogenetic abnormality on metaphase chromosome analysis. In a separate microarray data set (25), *BAP1* mRNA expression was reduced significantly in CD34<sup>+</sup> cells from MDS patients as compared to healthy controls (fig. S15), which is in keeping with *BAP1* being a tumor suppressor. To mimic *BAP1* haploinsufficiency, we characterized heterozygous *Bap1*<sup>fl/+</sup> creERT2<sup>+</sup> mice after tamoxifen treatment. Loss of one copy of *Bap1* caused very mild, but progressive, hema-

tological defects (fig. S16), which is important because the frameshift mutation identified in the patient is heterozygous.

Our results identify a previously unknown and potent tumor suppressor function for BAP1 in myeloid neoplasia. The BAP1 ortholog in *Drosophila*, called Calypso, suppresses *hox* gene expression (17), but we did not see increased *Hox* gene expression in BAP1 KO cells (fig. S17). Divergent epigenetic functions for fly and vertebrate BAP1 might reflect the fact that the HCF-1 binding motif conserved in vertebrate BAP1 (16, 20) is absent from Calypso. We propose that BAP1 forms a core complex with HCF-1 and OGT that can differentially recruit additional histone-modifying enzymes to regulate gene expression and thereby preserve normal hematopoiesis. It will be interesting to determine whether *Bap1* deficiency restricted to nonhematopoietic mouse tissues also promotes tumor development.

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#### Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1221711/DC1  
Materials and Methods  
Figs. S1 to S17  
References

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## Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga

Anne W. Thompson,<sup>1\*</sup> Rachel A. Foster,<sup>2\*</sup> Andreas Krupke,<sup>2</sup> Brandon J. Carter,<sup>1</sup> Niculina Musat,<sup>2†</sup> Daniel Vaultot,<sup>3</sup> Marcel M. M. Kuypers,<sup>2</sup> Jonathan P. Zehr<sup>1‡</sup>

Symbioses between nitrogen (N)<sub>2</sub>-fixing prokaryotes and photosynthetic eukaryotes are important for nitrogen acquisition in N-limited environments. Recently, a widely distributed planktonic uncultured nitrogen-fixing cyanobacterium (UCYN-A) was found to have unprecedented genome reduction, including the lack of oxygen-evolving photosystem II and the tricarboxylic acid cycle, which suggested partnership in a symbiosis. We showed that UCYN-A has a symbiotic association with a unicellular prymnesiophyte, closely related to calcifying taxa present in the fossil record. The partnership is mutualistic, because the prymnesiophyte receives fixed N in exchange for transferring fixed carbon to UCYN-A. This unusual partnership between a cyanobacterium and a unicellular alga is a model for symbiosis and is analogous to plastid and organismal evolution, and if calcifying, may have important implications for past and present oceanic N<sub>2</sub> fixation.

Nitrogen (N) is a primary nutrient whose availability constrains the productivity of the biosphere (1). Some bacteria and archaea can fix N<sub>2</sub> into biologically available ammonium and are important in the N cycle of terrestrial ecosystems and the global ocean. Although photosynthetic carbon (C) fixation evolved in eukaryotes through endosymbiosis of cyanobacteria that resulted in the chloroplast, no N<sub>2</sub>-fixing plastids or N<sub>2</sub>-fixing eukaryotes are known.

Nonetheless, N<sub>2</sub>-fixing symbioses are common in terrestrial environments between bacteria or cyanobacteria and multicellular plants. In the oceans, there are microscopic observations of probable symbioses between N<sub>2</sub>-fixing cyanobacteria and single-celled eukaryotic algae (2), although the nature of the interactions, if any, are unclear, except between the heterocystous N<sub>2</sub>-fixing cyanobacteria and their associated diatoms (3).

Recently, a geographically widespread uncultivated diazotrophic cyanobacterium (UCYN-A) (4) was found to have an unusual degree of genomic streamlining suggestive of obligate symbiosis. The streamlined genome of UCYN-A (1.44 million base pairs) lacks photosystem II (PS II: the oxygen-evolving component of the photosynthetic apparatus), RuBisCo (ribulose-1,5-bisphosphate carboxylase-oxygenase that fixes CO<sub>2</sub>), and the tricarboxylic acid cycle (TCA), features that usually define cyanobacteria (5, 6). UCYN-A requires organic carbon for energy (although it may obtain some energy through cyclic photophosphorylation around PS I) and biosynthesis, as well as a number of specific amino acids and nucleotides (5, 6). We propose the name *Candidatus Atelocyanobacterium thalassa* for UCYN-A. Dissolved organic carbon concentrations in the ocean are typically low, particularly for labile compounds such as glucose that UCYN-A would require (it has a complete gly-

<sup>1</sup>Ocean Sciences, University of California, Santa Cruz, CA 95064, USA. <sup>2</sup>Max-Planck-Institut für Marine Mikrobiologie, Bremen, Germany D-28359. <sup>3</sup>UPMC (Paris-06) and CNRS, UMR 7144, Station Biologique, Place G. Tessier 29680 Roscoff, France.

\*These authors contributed equally to this work.

†Present address: Department of Isotope Biogeochemistry, UFZ-Helmholtz Centre for Environmental Research, Leipzig 04318, Germany.

‡To whom correspondence should be addressed. E-mail: zehrj@ucsc.edu



## Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga

Anne W. Thompson *et al.*  
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(Fig. 4F) fits with their being recruited to promoters as a complex.

Given that BAP1 KO mice develop MDS, we investigated whether *BAP1* mutations occur in human MDS by full-length resequencing of *BAP1* in 32 paired tumor and normal samples from patients with de novo MDS. We identified a patient with a frameshift mutation that causes premature termination within the UCH catalytic domain of BAP1. Analysis of matched normal DNA did not identify the frameshift allele, which is consistent with somatic acquisition of the frameshift *BAP1* mutation by the MDS clone (fig. S14). The patient with the somatic *BAP1* mutation presented with refractory cytopenias and multilineage dysplasia, similar to the multilineage dysplasia and cytopenias seen in our murine model. Mutational profiling revealed that this patient was WT for known MDS mutations, including *TET2*, *ASXL1*, *EZH2*, *NRAS*, *C-KIT*, *FLT3*, *IDH1*, and *IDH2*, and had del(20)(q11.2q13.3) as the sole cytogenetic abnormality on metaphase chromosome analysis. In a separate microarray data set (25), *BAP1* mRNA expression was reduced significantly in CD34<sup>+</sup> cells from MDS patients as compared to healthy controls (fig. S15), which is in keeping with *BAP1* being a tumor suppressor. To mimic *BAP1* haploinsufficiency, we characterized heterozygous *Bap1*<sup>fl/+</sup> creERT2<sup>+</sup> mice after tamoxifen treatment. Loss of one copy of *Bap1* caused very mild, but progressive, hema-

tological defects (fig. S16), which is important because the frameshift mutation identified in the patient is heterozygous.

Our results identify a previously unknown and potent tumor suppressor function for BAP1 in myeloid neoplasia. The BAP1 ortholog in *Drosophila*, called Calypso, suppresses *hox* gene expression (17), but we did not see increased *Hox* gene expression in BAP1 KO cells (fig. S17). Divergent epigenetic functions for fly and vertebrate BAP1 might reflect the fact that the HCF-1 binding motif conserved in vertebrate BAP1 (16, 20) is absent from Calypso. We propose that BAP1 forms a core complex with HCF-1 and OGT that can differentially recruit additional histone-modifying enzymes to regulate gene expression and thereby preserve normal hematopoiesis. It will be interesting to determine whether *Bap1* deficiency restricted to nonhematopoietic mouse tissues also promotes tumor development.

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#### Supplementary Materials

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Materials and Methods  
Figs. S1 to S17  
References

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## Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga

Anne W. Thompson,<sup>1\*</sup> Rachel A. Foster,<sup>2\*</sup> Andreas Krupke,<sup>2</sup> Brandon J. Carter,<sup>1</sup> Niculina Musat,<sup>2†</sup> Daniel Vaultot,<sup>3</sup> Marcel M. M. Kuypers,<sup>2</sup> Jonathan P. Zehr<sup>1‡</sup>

Symbioses between nitrogen (N)<sub>2</sub>-fixing prokaryotes and photosynthetic eukaryotes are important for nitrogen acquisition in N-limited environments. Recently, a widely distributed planktonic uncultured nitrogen-fixing cyanobacterium (UCYN-A) was found to have unprecedented genome reduction, including the lack of oxygen-evolving photosystem II and the tricarboxylic acid cycle, which suggested partnership in a symbiosis. We showed that UCYN-A has a symbiotic association with a unicellular prymnesiophyte, closely related to calcifying taxa present in the fossil record. The partnership is mutualistic, because the prymnesiophyte receives fixed N in exchange for transferring fixed carbon to UCYN-A. This unusual partnership between a cyanobacterium and a unicellular alga is a model for symbiosis and is analogous to plastid and organismal evolution, and if calcifying, may have important implications for past and present oceanic N<sub>2</sub> fixation.

Nitrogen (N) is a primary nutrient whose availability constrains the productivity of the biosphere (1). Some bacteria and archaea can fix N<sub>2</sub> into biologically available ammonium and are important in the N cycle of terrestrial ecosystems and the global ocean. Although photosynthetic carbon (C) fixation evolved in eukaryotes through endosymbiosis of cyanobacteria that resulted in the chloroplast, no N<sub>2</sub>-fixing plastids or N<sub>2</sub>-fixing eukaryotes are known.

Nonetheless, N<sub>2</sub>-fixing symbioses are common in terrestrial environments between bacteria or cyanobacteria and multicellular plants. In the oceans, there are microscopic observations of probable symbioses between N<sub>2</sub>-fixing cyanobacteria and single-celled eukaryotic algae (2), although the nature of the interactions, if any, are unclear, except between the heterocystous N<sub>2</sub>-fixing cyanobacteria and their associated diatoms (3).

Recently, a geographically widespread uncultivated diazotrophic cyanobacterium (UCYN-A) (4) was found to have an unusual degree of genomic streamlining suggestive of obligate symbiosis. The streamlined genome of UCYN-A (1.44 million base pairs) lacks photosystem II (PS II: the oxygen-evolving component of the photosynthetic apparatus), RuBisCo (ribulose-1,5-bisphosphate carboxylase-oxygenase that fixes CO<sub>2</sub>), and the tricarboxylic acid cycle (TCA), features that usually define cyanobacteria (5, 6). UCYN-A requires organic carbon for energy (although it may obtain some energy through cyclic photophosphorylation around PS I) and biosynthesis, as well as a number of specific amino acids and nucleotides (5, 6). We propose the name *Candidatus Atelocyanobacterium thalassa* for UCYN-A. Dissolved organic carbon concentrations in the ocean are typically low, particularly for labile compounds such as glucose that UCYN-A would require (it has a complete gly-

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†Present address: Department of Isotope Biogeochemistry, UFZ-Helmholtz Centre for Environmental Research, Leipzig 04318, Germany.

‡To whom correspondence should be addressed. E-mail: zehrj@ucsc.edu



colysis pathway). Because UCYN-A has a complete suite of nitrogenase genes and related genes required for nitrogen fixation, it was hypothesized that UCYN-A provides fixed nitrogen in exchange for fixed carbon from a symbiotic partner (5, 7).

We tested different possible partner phytoplankton populations in seawater samples from the North Pacific Ocean (Fig. 1, fig. S1, and table S1) for the presence of symbiotic UCYN-A by screening flow-cytometrically sorted cells with a UCYN-A-specific quantitative PCR (qPCR) assay for the nitrogenase gene (*nifH*) (8). The UCYN-A genome had been obtained with this approach (6), but the seawater was first concentrated by vacuum filtration before sorting, which dislodged the UCYN-A from their associated cells (fig. S1). Here, we used a similar flow sorting procedure, but instead, raw seawater that had not been preserved or concentrated was immediately sorted and resulted in detection of most (63 to 94%) of the UCYN-A *nifH* genes in the sorted photosynthetic picoeukaryote population (PPE) (1- to 3- $\mu$ m-diameter cells) rather than other pigmented and nonpigmented cells (sorted populations displayed in Fig. 1). The data unequivocally showed that UCYN-A is associated with photosynthetic picoeukaryotic cells. These results explain the reports of UCYN-A *nifH* in filter-fractionated samples from the California Coast (0.8- to 3- $\mu$ m and 3- to 200- $\mu$ m fractions) (9) and Station ALOHA (1- to 3- $\mu$ m fraction) (5). We now only observe an enriched population of free UCYN-A cells (0.2 to 1  $\mu$ m) after seawater concentration by vacuum filtration, freezing for storage purposes, and resuspension in sterile seawater, which apparently disrupts the fragile association (fig. S1). UCYN-A appears to be in a loose extracellular association (epiphytic) that

is easily dislodged, which explains why there is some amplification of UCYN-A *nifH* from outside the region of the sorted photosynthetic picoeukaryote population (table S1). This delicate association is similar to microscopic observations of other probable marine plankton symbioses, including the mixed populations of unicellular *Synechococcus*- and *Crocosphaera*-like unicellular cyanobacteria housed in the girdle of *Dinophysis* (dinoflagellates) (10).

The marine picoeukaryotic population defined by flow cytometry is extremely diverse (11–13). Therefore, to identify the specific cells associated with UCYN-A, we compared universal 18S ribosomal RNA (rRNA) and 16S rRNA gene clone libraries that were amplified from sorted samples of the entire picoeukaryote population to those from sorted single picoeukaryote cells (table S1). Single cells and the entire picoeukaryote population were initially screened for UCYN-A by *nifH* qPCR. As expected, the partial 18S rRNA gene sequences [~730 base pairs (bp)] derived from the entire picoeukaryote population sorts were diverse and included sequences from several classes of marine picoeukaryotes (Fig. 2 and table S3). 16S rRNA gene sequences amplified from the entire picoeukaryote population sorts were also diverse and confirmed the presence of UCYN-A in these populations (table S3). However, amplification of the 18S rRNA gene (using nested PCR) from single UCYN-A *nifH*-positive sorted picoeukaryote cells yielded exclusively prymnesiophyte sequences (Fig. 2 and table S4).

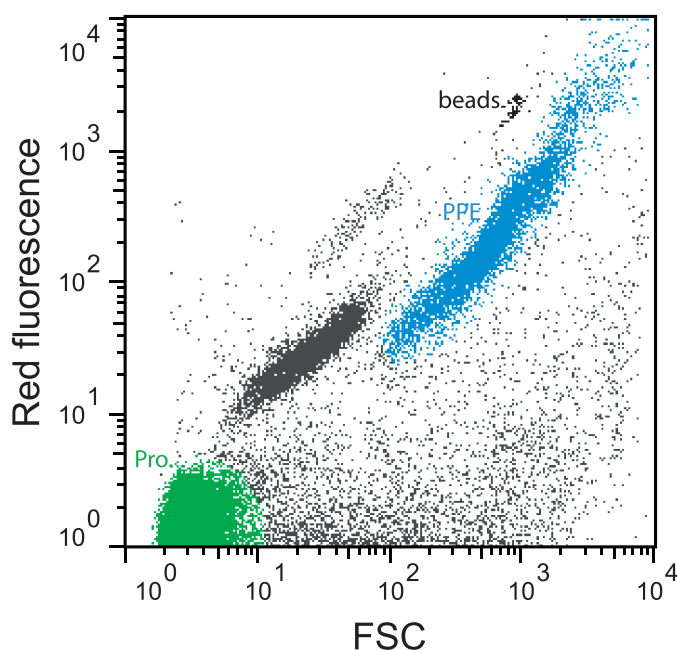
The partial 18S rRNA gene sequences [or “partner” sequences from the Ek555/1269 primer amplicon, GenBank accession nos. JX291679 to JX291804 and JX291547 to JX291678], derived from 12 single UCYN-A-*nifH*-positive picoeu-

karyotic cells, were greater than 99.5% identical to each other and had best BLASTn hits (>99% identical) to a sequence derived from sorted picoplankton from the oligotrophic South Pacific Ocean (GenBank accession no. FJ537341, BIOSOPE T60.34, sample T60, Station STB11, -107.29°E, -27.77°S) (14) (Fig. 2). A metagenome from sorted photosynthetic picoeukaryotes of the same sample (BIOSOPE, sample T60) also contained numerous DNA sequence reads (mean length of 340 bp) that were almost identical (99 to 100%) to the UCYN-A genome. Samples from an adjacent station (STB7, samples T39 and T40) contained neither good matches to the UCYN-A genome nor the partner partial 18S rRNA gene sequence we identified (table S2 and fig. S2). Assembly of the UCYN-A reads from sample T60 covered 12.4% of the UCYN-A genome (table S2), providing additional evidence that UCYN-A is associated with the picoeukaryotes and that the UCYN-A partner is the same in the oligotrophic North and South Pacific Oceans.

We examined the phylogeny of the full-length 18S rRNA gene of the BIOSOPE environmental sequence (T60.34) (UCYN-A partner sequence best BLASTn hit) relative to the diverse prymnesiophyte class (11, 15, 16). The BIOSOPE T60.34 sequence clustered with the calcareous nanoplankton *Braarudosphaera bigelowii* (17–20) and with *Chrysochromulina parkeae* (21, 22), which may contain calcified scales as well (23) (Fig. 2). *B. bigelowii* appears to represent several pseudo-cryptic species that are all easily differentiated from other calcareous phytoplankton by their distinct pentagonal plates (19). Production of calcified plates by the UCYN-A partner is an intriguing possibility, because calcareous phytoplankton are important in the vertical flux of carbon and nitrogen in the oceans. Sedimentary records from the late Cretaceous show fossils of *Braarudosphaera* species in open ocean sediments (24, 25), suggesting that the UCYN-A symbiosis could be ancient and could potentially be studied in a paleo-oceanographic context. However, prymnesiophytes (for example, *Emiliania huxleyi*) have complex life histories in which form (in particular calcification) and behavior change dramatically between haploid and diploid life stages [references in (26)]. Because the life-history stage of the partner cells in the natural populations of this study is unknown and sample processing could have dissolved or removed plates, whether or not the partner is calcifying could not be determined. Calcification of the partner cell could be an important facet in its symbiosis with UCYN-A, because it could provide a mechanism for stabilizing an extracellular association.

Prymnesiophytes are typically free-living and photosynthetic and therefore could provide organic C for an associated photoheterotroph like UCYN-A. To test this hypothesis, we applied a halogenated in situ hybridization nanometer-scale secondary ion mass spectrometry (HISH-SIMS) approach to natural phytoplankton populations

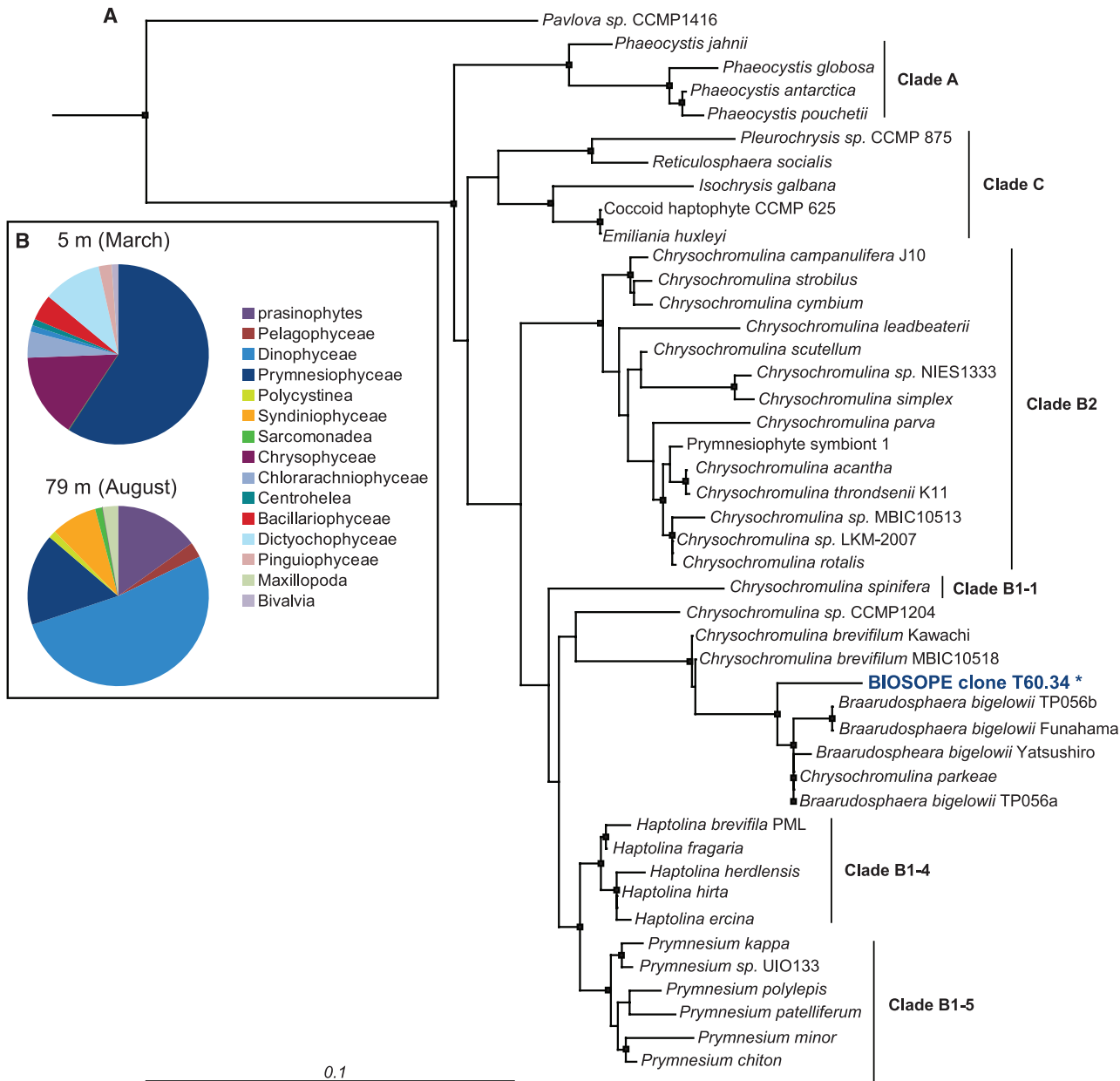
**Fig. 1.** Example flow cytogram of cell populations in unpreserved seawater that were targeted in this study. Red fluorescence is a measure of chlorophyll a concentration per cell. Forward scatter (FSC) is a proxy for cell size. Beads 3  $\mu$ m in diameter (black) were used for reference. Cell populations indicated are photosynthetic picoeukaryotes (PPE, blue), *Prochlorococcus* (*Pro.*, green), and cells (gray) that are not PPE or *Pro.* Coloring of each population indicates the sort gates used. Most UCYN-A *nifH* gene copies (63 to 94%) were amplified from the PPE population (table S1). Flow cytograms of all samples used in this study are presented in fig. S1.



from Station ALOHA (27) that were amended with  $^{15}\text{N}_2$  and  $^{13}\text{C}$ -bicarbonate ( $\text{H}^{13}\text{CO}_3$ ) and incubated under in situ conditions. The photosynthetic picoeukaryote cells (diameter 1 to 3  $\mu\text{m}$ ) were subsequently sorted by flow cytometry, preserved, and processed for HISH-SIMS (27, 28). A highly specific oligonucleotide probe for the UCYN-A 16S rRNA gene (UCYN-732) (27) confirmed the presence of 278 UCYN-A cells among the sorted photosynthetic picoeukaryote population. Most (163, 59%) of the UCYN-A cells (diameter 0.31 to 0.92  $\mu\text{m}$ ) remained associated with a larger

partner cell (diameter 0.99 to 1.76  $\mu\text{m}$ ) during sample processing. UCYN-A cells were mainly observed at one end of the partner cell in what appeared to be an indentation (Fig. 3A). Numerous UCYN-A cells (107, 38%) were dislodged from their eukaryotic partners during HISH-SIMS sample processing (the cells were initially attached, otherwise they would not have been sorted by flow cytometry) and observed without an associated cell. Some (3%) of the UCYN-A cells identified by HISH were present as pairs located at opposite ends of a single partner cell (Fig. 3A). In other

planktonic symbioses involving cyanobacteria (e.g., diatom symbionts with filamentous heterocyst-forming cyanobacteria), the cyanobacteria migrate to polar ends of the larger partner cells before cell division of the host (29). Similarly, the partner cells with multiple associated UCYN-A cells may have been dividing, because the incubation period was longer than a typical cell division cycle (36 hours). Measurements of scanning electron micrographs (SEMs) of individual *B. bigelowii* cells from enrichments of coastal waters show three size classes, the smallest of which corresponds



**Fig. 2.** Diversity and phylogeny of UCYN-A *nifH*-positive sorted picoeukaryote populations and single cells from 5-m and 79-m depths at Station ALOHA. **(A)** Maximum-likelihood tree (PhyML) of selected cultured prymnesiophyte 18S rRNA gene sequences with clade assignments and genus names per (15). "BIOSOPE T60.34" sequence (blue type and asterisk) is the best BLASTn hit of the UCYN-A partner sequence amplified

from UCYN-A *nifH*-positive single photosynthetic picoeukaryotes. Node support greater than 75% is marked by black squares. **(B)** 18S rRNA gene diversity of the entire sorted picoeukaryote population that was the source of single cell sorts from March (5-m depth, cruise KM1110) and August (79-m depth, cruise HOT234) that were positive for UCYN-A *nifH* and the BIOSOPE T60.34 sequence.

to cell diameters of about 5  $\mu\text{m}$ , which is larger than the UCYN-A partner cell measured here (18, 19). If calcareous plates are present on the UCYN-A partner, loss of the plates during sample processing for HISH-SIMS may result in measurement of a smaller cell diameter than cells measured by SEM. Alternatively, the UCYN-A partner may represent an additional smaller-size class of *B. bigelowii* adapted to the oligotrophic ocean.

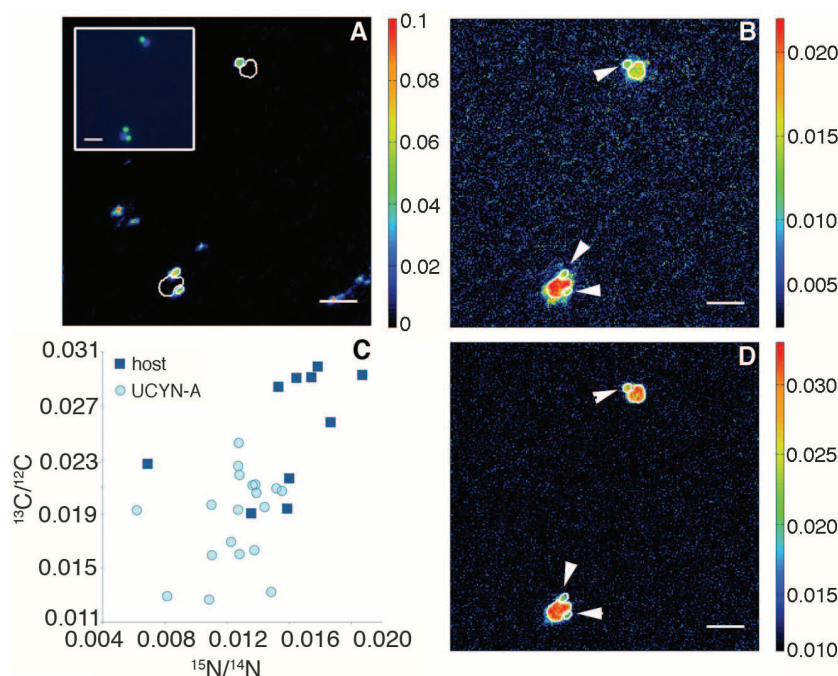
Ten partner cells and their associated UCYN-A cells were chosen for quantitative isotopic analysis with nanoSIMS. On average,  $^{13}\text{C}/^{12}\text{C}$  enrichment was lower in the UCYN-A cells than in the partner cells (average  $^{13}\text{C}$  atom % of 1.8139 and 2.4602, respectively; Fig. 3, B and C, and table S6). Because the UCYN-A genome does not contain carbon-fixation pathways (5, 6), but photosynthetic picoeukaryotes do, we conclude that the  $^{13}\text{C}$  enrichment in UCYN-A was due to transfer of fixed C from the eukaryotic partner. Both partner and UCYN-A were strongly  $^{15}\text{N}$ -enriched (Fig. 3, C and D, and table S6). As only some bacteria and some archaea can fix  $\text{N}_2$ , our nanoSIMS data provide direct evidence for active  $\text{N}_2$  fixation by the uncultivated UCYN-A. The average  $^{15}\text{N}/^{14}\text{N}$  enrichment was higher in the partner cells than in the UCYN-A cells (average  $^{15}\text{N}$  atom % of 1.5308 and 1.2081, respectively), showing that extensive amounts (we estimate up

to 95%) of fixed N were transferred from UCYN-A to the partner cell. In contrast, little of the C (1 to 17%) fixed by the partner cell was transferred to the UCYN-A, consistent with the lower C requirements of a small slow-growing heterotrophic symbiont. The large fraction of N transferred to the partner is consistent with results from known symbioses, such as between heterocystous cyanobacteria and marine planktonic diatoms (3).

The symbiosis reported here is unusual in that it is a partnership between a prymnesiophyte and a unicellular cyanobacterium. The results provide an explanation for how the metabolically streamlined UCYN-A survives in the oligotrophic ocean, despite the lack of the TCA cycle, PS II, and some biosynthetic pathways. Tracer experiments using  $^{15}\text{N}$  and  $^{13}\text{C}$  clearly show that the cyanobacterium provides fixed N to the eukaryotic partner and conversely that C fixation by the eukaryotic partner can provide C to UCYN-A. Thus, the association appears to be at least a mutualistic and facultative, if not an obligate, association. It is still not known whether the cyanobacterium is an endosymbiont or lives on the surface of the prymnesiophyte. However, the sensitivity to disruption and the HISH-SIMS imaging indicates that it is a loose cell-surface association. Many of the dislodged cells observed after HISH-SIMS were located near a picoeukaryotic cell.

Because *B. bigelowii*, the closest known relative of the sequence amplified from our single-cell sorts, has calcareous plates that are easily dislodged, it is conceivable that UCYN-A is somehow associated with these plates. Sample handling and processing, in particular the HISH assay, could have dislodged or dissolved the calcium carbonate plates, releasing UCYN-A.

The association of UCYN-A with prymnesiophytes suggests that N fixed by UCYN-A may enter the microbial loop through this group of relatively abundant and globally relevant primary producers and mixotrophs (11, 16, 30, 31) and has other important implications. The UCYN-A partner may be calcifying, which has implications for the contributions of  $\text{N}_2$ -based new production to vertical carbon fluxes (32), the sensitivity of the UCYN-A partner to ocean acidification (33), and paleo-oceanographic microfossils in sediments. Equally interesting is the existence of a planktonic unicellular  $\text{N}_2$ -fixing symbiosis and its implications for evolution and adaptation. The presence of these simple interactions between single-celled organisms are reminiscent of those earlier primary endosymbiotic events and underscores the enigmatic absence of  $\text{N}_2$ -fixing plastids in evolution as  $\text{N}_2$  fixation is an energetically expensive oxygen-sensitive reaction, and nitrogenase is an ancient enzyme. Thus, the UCYN-A association is a symbiosis with a prymnesiophyte and provides an intriguing model for the evolution of  $\text{N}_2$  fixation, and the mutualistic interactions between planktonic microorganisms.



**Fig. 3.** Microscopy and elemental composition of two UCYN-A partner cells and their associated UCYN-A cells detected in samples from sorted picoeukaryotes analyzed by HISH-SIMS. (A)  $^{19}\text{F}/^{12}\text{C}$  (HISH) labeling of UCYN-A. Inset displays labeling of the same UCYN-A cells by catalyzed reporter deposition-fluorescence in situ hybridization (green) and DAPI (4',6-diamidino-2-phenylindole) staining of partner cell nucleus (blue). (B) The  $^{13}\text{C}/^{12}\text{C}$  ratio image of UCYN-A and partner cell. (C) The  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  in 10 selected partner cells and their associated UCYN-A cells (table S6). (D) The  $^{15}\text{N}/^{14}\text{N}$  image ratio of UCYN-A and partner cell. The white lines define regions of interest that were used for calculating  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratios. UCYN-A cells are indicated by white arrows in (B) and (D). Scale bar, 3  $\mu\text{m}$ .

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# Supplementary Materials

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Materials and Methods  
Figs. S1 and S2  
Tables S1 to S6  
References (34–46)

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# Disruption of Reconsolidation Erases a Fear Memory Trace in the Human Amygdala

Thomas Agren,<sup>1</sup> Jonas Engman,<sup>1</sup> Andreas Frick,<sup>1</sup> Johannes Björkstrand,<sup>1</sup> Elna-Marie Larsson,<sup>2</sup> Tomas Furmark,<sup>1</sup> Mats Fredrikson<sup>1</sup>

Memories become labile when recalled. In humans and rodents alike, reactivated fear memories can be attenuated by disrupting reconsolidation with extinction training. Using functional brain imaging, we found that, after a conditioned fear memory was formed, reactivation and reconsolidation left a memory trace in the basolateral amygdala that predicted subsequent fear expression and was tightly coupled to activity in the fear circuit of the brain. In contrast, reactivation followed by disrupted reconsolidation suppressed fear, abolished the memory trace, and attenuated fear-circuit connectivity. Thus, as previously demonstrated in rodents, fear memory suppression resulting from behavioral disruption of reconsolidation is amygdala-dependent also in humans, which supports an evolutionarily conserved memory-update mechanism.

Anxiety disorders are common, and they cause great suffering and high societal costs (1). The etiology involves amygdala-dependent memory mechanisms that link stressful events to previously neutral stimuli (2), and the amygdala has been demonstrated to be hyperresponsive across the anxiety disorders (3). Pharmacological and behavioral treatments of anxiety reduce symptomatology and amygdala activity (4) but have limited success because relapses occur (5). However, fear memories may be erased by recalling them and preventing their reconsolidation (6, 7). In rodents, the amygdala seems vital for fear memory reconsolidation (7, 8), but this has not been investigated in humans.

Fear conditioning, in which a previously neutral stimulus turns into a conditioned stimulus

(CS) through pairings with an aversive stimulus, forms a memory trace in the amygdala (2). Memory activation produces behavioral (2, 9) and autonomic fear reactions, such as skin conductance responses (SCRs) (10–12), frequently used to measure fear learning. Studies in animals (13) and anxiety patients (14) demonstrate that extinction weakens, but does not erase, fear memories. In rodents (13) and humans (15) alike, extinction attenuates conditioned fear expression through prefrontal inhibition. Fear can return after stress, be renewed when altering the environmental context, or reoccur with the passage of time (16).

By activating memories and disrupting their reconsolidation, through protein synthesis blockade local in the amygdala (8) or through systemic administration of  $\beta$ -adrenergic receptor antagonists (17, 18), fear memories are inhibited. Fear memory reconsolidation can also be disrupted behaviorally (6, 7, 19). In rodents, extinction of fear conditioning performed 10 or 60 min after presenting a reminder of the conditioned fear, but not after 6 or 24 hours, inhibited fear expression (7). Fear did not return in a new context, after

shock exposure, or with time. Thus, extinction conducted within, but not outside, the reconsolidation window resulted in permanent attenuation of the fear memory (7).

In humans, extinction performed within the reconsolidation interval also inhibited fear, whereas extinction training performed outside of the reconsolidation interval spared the memory and fear returned (6). In animals, the neural functions enabling fear memory formation and reconsolidation are located in the amygdala (2, 7–9, 20). In humans, lesion (21) and brain imaging studies (10–12, 22) confirm that the amygdala is a key area for fear memory encoding. To test the hypothesis that reconsolidation in humans is amygdala-mediated and that disruption of reconsolidation inactivates a memory trace in the basolateral amygdala, we performed a study combining brain imaging with a physiological measure of fear.

On day 1, twenty-two subjects (11 women) aged  $24.0 \pm 0.48$  (mean  $\pm$  SEM) underwent fear conditioning to establish an associative fear memory (Fig. 1A and fig. S1). On day 2, the fear memory was reactivated by presenting the cue previously paired with the shock (CS+) for 2 min. Subjects were randomized into two groups. One group received extinction, consisting of repeated CS presentations with the shock withheld, 10 min after reactivation and thus within the reconsolidation interval. The other group received extinction 6 hours after the reactivation—i.e., outside of the interval. Fear expression was measured using SCRs (6, 19). On day 3, a renewal session was performed in a new environment, a magnetic resonance scanner, where shock electrodes were attached, although no shocks were delivered. SCRs were not measured for technical reasons. On day 5, subjects were exposed to unsignaled shocks and then re-exposed to CS+. Return of fear was defined as the increase in SCR from the last extinction trial on day 2 to the first reinstatement trial on day 5 (Fig. 1B) (6).

First, we evaluated if the predicted behavioral reinstatement effect was present on day 5. Confirming this, increased fear responding was observed in the 6 hours, but not the 10 min group

<sup>1</sup>Department of Psychology, Uppsala University, SE-751 42 Uppsala, Sweden. <sup>2</sup>Department of Radiology, Oncology and Radiation Science, Uppsala University, SE-751 42 Uppsala, Sweden.

\*To whom correspondence should be addressed. E-mail: thomas.agren@psyk.uu.se

(Fig. 1B, right panel). Groups were indistinguishable in acquisition and extinction (Fig. 1B and fig. S1).

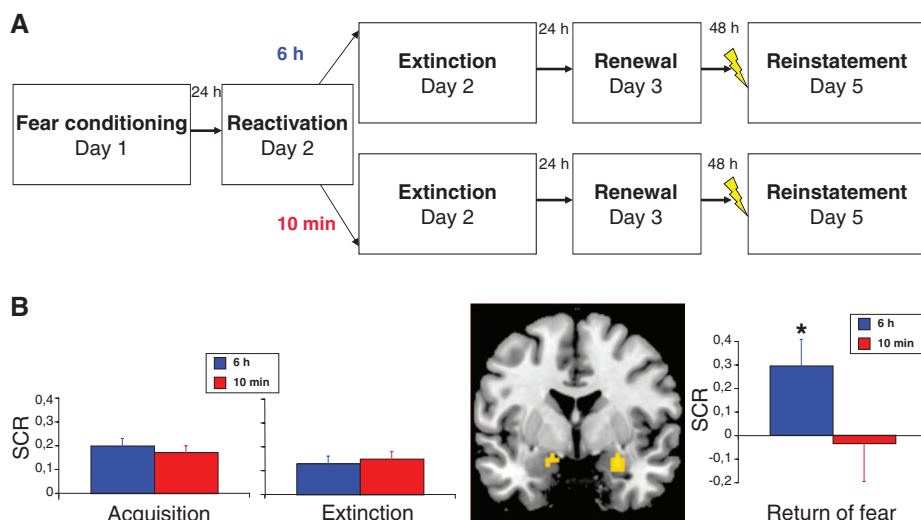
Next, we tested the hypothesis that the fear memory representation is localized to the amygdala. Significantly greater activity was evident bilaterally in the basolateral amygdala in the

6 hours group as compared with the 10 min group (Fig. 1B).

We then tested if the amygdala-localized memory predicted return of fear. Positive correlations were present between return of fear and blood oxygen level-dependent (BOLD) activity bilaterally in the basolateral amygdala in the 6 hours

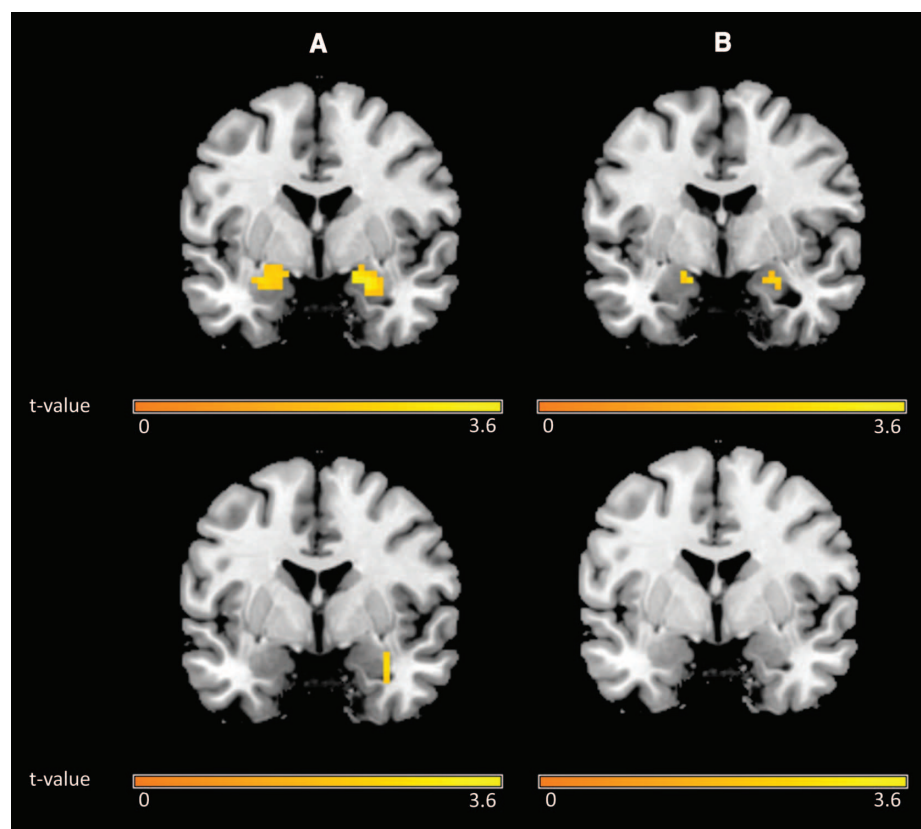
group (Fig. 2A). In the 10 min group, a cluster in the right claustrum extending into the amygdala correlated significantly with SCRs (Fig. 2A). BOLD activity reflecting the amygdala-localized memory trace also correlated with fear recall during extinction the previous day in the 6 hours, but not the 10 min, group (Fig. 2B).

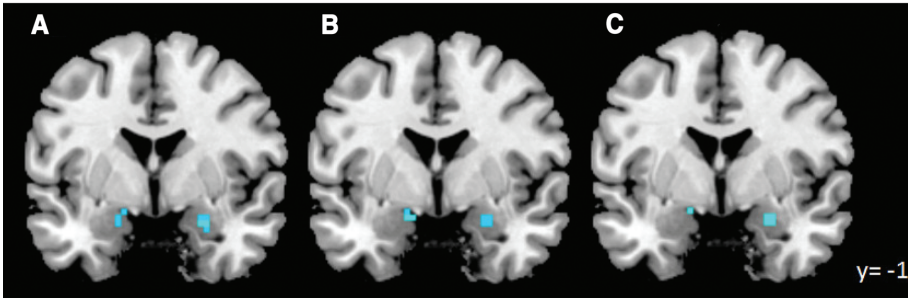
**Fig. 1.** Extinction during reconsolidation blocks reinstatement of fear and abolishes a memory trace in the amygdala. **(A)** After fear conditioning on day 1, when 16 shocks were paired with a visual cue, a memory reminder was given on day 2, and extinction was performed after 10 min or 6 hours, by exposure to eight conditioned cues with no shocks. On day 3, amygdala activity was assessed with functional magnetic resonance imaging (fMRI) during renewal-induced fear. On day 5, return of fear was evoked by presenting unpaired shocks before CSs were again presented. **(B)** Groups were equivalent in acquisition [ $t(20) = 0.66$ ,  $P = 0.51$ ] and extinction [ $t(20) = 1.03$ ,  $P = 0.31$ ]. Return of fear was confirmed in the 6 hours group [blue bar;  $t(10) = 2.72$ ,  $P = 0.02$ ] but not in the 10 min group [red bar;  $t(8) = 0.23$ ,  $P = 0.82$ ]. fMRI demonstrated a remaining fear memory representation in the amygdala after reactivation and normal reconsolidation but not after reactivation followed by disrupted reconsolidation. The voxels reflecting the bilateral memory trace, encompassing the basolateral amygdala, indicate superior memory representation in the 6 hours as compared with the 10 min group (brain coordinates:  $x, y, z = 27, 5, -17$ ;  $Z$ -score = 2.46;  $P = 0.007$ ; 378 mm<sup>3</sup>;  $x, y, z = -15, -1, -14$ ;  $Z$ -score = 2.22;  $P = 0.013$ ; 162 mm<sup>3</sup>).



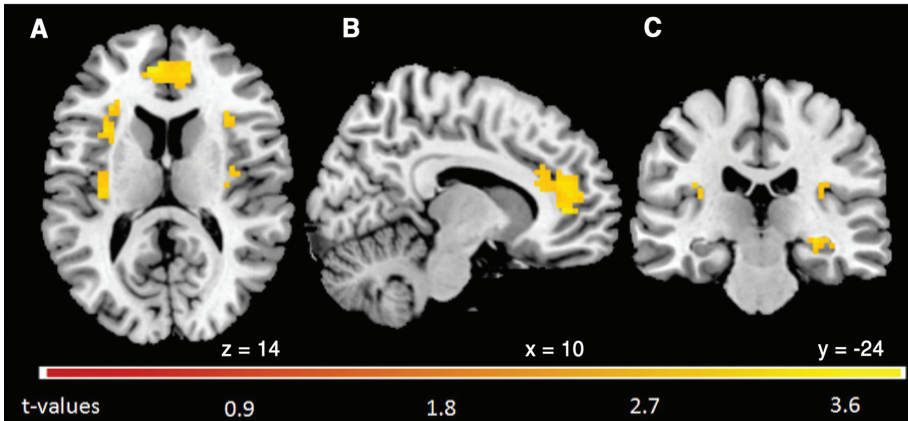
The autonomic nervous system measure of fear is the SCR. The CNS measure of amygdala activity is BOLD activity. Brain coordinates are according to the Montreal Neurological Institute (MNI). Error bars are standard error of means.

**Fig. 2.** Amygdala activity predicts return of fear and correlates with recall of fear. **(A)** In the 6 hours group (top), activity bilaterally in the basolateral amygdala predicted return of fear 2 days later ( $x, y, z = 21, -1, -17$ ;  $Z = 2.06$ ;  $P = 0.002$ ; 999 mm<sup>3</sup>;  $x, y, z = -21, -4, -14$ ;  $Z = 2.38$ ;  $P = 0.009$ ; 1107 mm<sup>3</sup>). In the 10 min group (bottom), an area in the right temporal claustrum extending into the amygdala was also related to SCR ( $x, y, z = 33, 2, -23$ ;  $Z = 2.49$ ;  $P = 0.006$ ; 324 mm<sup>3</sup>). Because fear did not return in the 10 min group, the correlation may reflect individual brain-behavior relations unrelated to fear and the experimental manipulation. **(B)** In the 6 hours group (top), recall of fear during extinction covaried with the strength of amygdala activity bilaterally ( $x, y, z = 24, -1, -20$ ;  $Z = 2.35$ ;  $P = 0.009$ ; 378 mm<sup>3</sup>;  $x, y, z = -15, 4, -17$ ;  $Z = 2.27$ ;  $P = 0.012$ ; 189 mm<sup>3</sup>). No covariation existed in the 10 min group (bottom).





**Fig. 3.** Areas in the amygdala that correlate with recall and return of fear are colocalized with the memory trace. (A) The amygdala areas reflecting the memory trace (Fig. 1) overlapped with the areas that predicted return of fear in the 6 hours group ( $x, y, z = 24, 2, -17$ ;  $189 \text{ mm}^3$ ;  $x, y, z = -15, -1, -14$ ;  $108 \text{ mm}^3$ ), illustrated in Fig. 2A (top). (B) The localization of the memory trace also overlapped with areas involved in recall during extinction ( $x, y, z = 24, 2, -20$ ;  $81 \text{ mm}^3$ ;  $x, y, z = -15, -4, -17$ ;  $135 \text{ mm}^3$ ) illustrated in Fig. 2B (top), in the 6 hours group only. (C) These three areas overlapped with each other ( $x, y, z = 24, 2, -20$ ;  $135 \text{ mm}^3$ ;  $x, y, z = -15, -1, -14$ ;  $27 \text{ mm}^3$ ).



**Fig. 4.** Amygdala memory trace activity is functionally coupled to the fear network in the brain after normal memory reconsolidation but not after disrupted reconsolidation. The connectivity analysis, using BOLD activity in the amygdala from areas representing memory trace activity in the 6 hours group as a seed of interest, demonstrated stronger functional couplings in the 6 hours than in the 10 min group in structures forming the fear-circuit of the brain, including the midline anterior cingulate cortex (A and B) ( $x, y, z = -9, 47, 13$ ;  $Z = 3.14$ ;  $5994 \text{ mm}^3$ ), bilateral insula (A and C) ( $x, y, z = 33, 20, 7$ ;  $Z = 3.28$ ;  $1890 \text{ mm}^3$ ;  $x, y, z = -27, 29, 10$ ;  $Z = 2.95$ ;  $4077 \text{ mm}^3$ ), and bilateral hippocampus (C), although only the right hippocampus is illustrated ( $x, y, z = -27, -13, -14$ ;  $Z = 2.51$ ;  $297 \text{ mm}^3$ ;  $x, y, z = 30, -25, -8$ ;  $Z = 2.51$ ; total  $675 \text{ mm}^3$ ).

Amygdala areas harboring the memory trace (Fig. 1B) and areas covarying with return of fear (Fig. 2A) overlapped in the 6 hours group only (Fig. 3A). Moreover, the memory trace was colocalized to areas involved in fear memory recall during extinction (Fig. 3B). Finally, all these areas overlapped with each other only in the 6 hours group (Fig. 3C). Thus, the localization of the memory trace in the amygdala overlapped bilaterally with areas related both to recall of fear during extinction and return of fear during reinstatement. The hypothesis that memory was not erased, but only suppressed, by extinction-mediated prefrontal inhibition was not supported because the theoretically predicted (13, 15) negative coupling between activity in the ventromedial prefrontal cortex (vmPFC) and return of fear was absent because vmPFC activity did not

correlate negatively with fear in either group ( $Z$ -scores of  $<1$ ).

Finally, we evaluated if activation of the fear memory in the amygdala was linked to activity in other nodes of the fear network (23) by calculating the covariation between memory-associated amygdala activity and activity in the remaining network. Our amygdala seed of interest correlated strongly with activity bilaterally in the insula, hippocampus, and the midline anterior cingulate cortex and significantly more so in the 6 hours than in the 10 min group (Fig. 4). No clusters showed a better correlation with the amygdala seed in the 10 min group. This suggests that the amygdala could be the primary site of memory plasticity, but also influence reconsolidation by affecting other regions of the fear network. The amygdala could thus play a modulatory, rather

than a solitary, role in human fear reconsolidation processes.

In summary, whereas the amygdala memory representation after activation and undisrupted reconsolidation predicted return of fear and was functionally coupled to other nodes of the brain's fear network, disruption of reconsolidation significantly weakened the amygdala memory and its coupling, rendering it unrelated to both recall and return of fear. We conclude that extinction training initiated during reconsolidation abolishes fear expression by erasing a memory trace in the amygdala. Reactivated fear memories are sensitive to behavioral disruption (6, 7, 19), and the amygdala proves to be a key neurobiological substrate for this process also in humans. This mechanism holds great clinical promise in anxiety treatment (6, 17–19) in order to dissociate fear from cognitive memory.

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#### Supplementary Materials

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Supplementary Text  
Fig. S1  
References (24–28)

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## Acute Gastrointestinal Infection Induces Long-Lived Microbiota-Specific T Cell Responses

Timothy W. Hand *et al.*  
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# Acute Gastrointestinal Infection Induces Long-Lived Microbiota-Specific T Cell Responses

Timothy W. Hand,<sup>1</sup> Liliane M. Dos Santos,<sup>1,2</sup> Nicolas Bouladoux,<sup>1</sup> Michael J. Molloy,<sup>1</sup> Antonio J. Pagán,<sup>3</sup> Marion Pepper,<sup>3,4</sup> Craig L. Maynard,<sup>5</sup> Charles O. Elson III,<sup>6</sup> Yasmine Belkaid<sup>1\*</sup>

The mammalian gastrointestinal tract contains a large and diverse population of commensal bacteria and is also one of the primary sites of exposure to pathogens. How the immune system perceives commensals in the context of mucosal infection is unclear. Here, we show that during a gastrointestinal infection, tolerance to commensals is lost, and microbiota-specific T cells are activated and differentiate to inflammatory effector cells. Furthermore, these T cells go on to form memory cells that are phenotypically and functionally consistent with pathogen-specific T cells. Our results suggest that during a gastrointestinal infection, the immune response to commensals parallels the immune response against pathogenic microbes and that adaptive responses against commensals are an integral component of mucosal immunity.

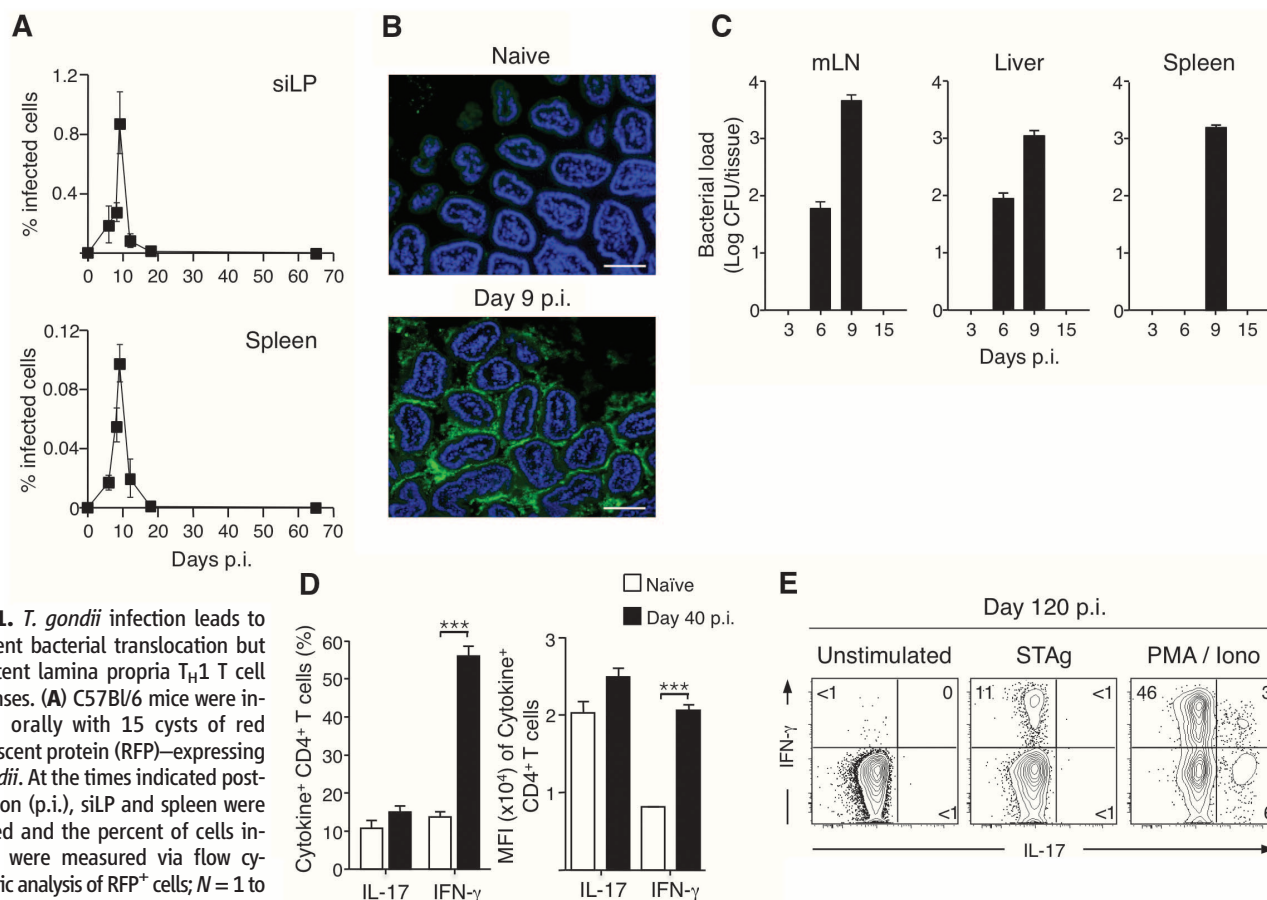
The intestinal microbiome is essential to multiple aspects of host physiology (1). Recent studies have estimated that the hu-

man microbiome contains  $\sim 3 \times 10^6$  distinct genes each of which may possess multiple antigens (2). Regulating immune responses to this extraordi-

narily diverse set of antigens is a formidable task because commensals also express inflammatory pathogen-associated molecular patterns (PAMPs) that could potentially activate the host immune response (3). To limit contact with the commensal microbiota, the gut is compartmentalized and contains various innate and adaptive mechanisms that prevent adaptive immune responses against food and commensal antigens (4–6). Maintaining

<sup>1</sup>Mucosal Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health (NIH), Bethesda, MD 20892, USA. <sup>2</sup>Laboratory of Gnotobiology and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais 31270-901, Brazil. <sup>3</sup>Microbiology, Immunology and Cancer Biology Program, University of Minnesota, Minneapolis, MN 55455, USA. <sup>4</sup>Department of Immunology, University of Washington, Seattle, WA 98195, USA. <sup>5</sup>Department of Pathology, University of Alabama–Birmingham, Birmingham, AL 35294, USA. <sup>6</sup>Mucosal HIV and Immunobiology Center, University of Alabama–Birmingham, Birmingham, AL 35294, USA.

\*To whom correspondence should be addressed. E-mail: ybelkaid@niaid.nih.gov



**Fig. 1.** *T. gondii* infection leads to transient bacterial translocation but persistent lamina propria  $T_H1$  T cell responses. (A) C57BL/6 mice were infected orally with 15 cysts of red fluorescent protein (RFP)-expressing *T. gondii*. At the times indicated post-infection (p.i.), siLP and spleen were isolated and the percent of cells infected were measured via flow cytometric analysis of RFP<sup>+</sup> cells;  $N = 1$  to 8 independent experiments,  $n = 3$  to 15 mice per time point. (B) Small intestinal samples were isolated from naive and day 9 *T. gondii*-infected mice. Intestinal samples were then fixed, sectioned, counter-stained with 4',6-diamidino-2-phenylindole (DAPI) and hybridized for FISH with a fluorescent probe specific to eubacterial 16S chromosomal sequences. Scale bar, 25  $\mu$ m;  $N = 7$  independent experiments. (C) Counts of bacterial colonies cultured from spleen, mesenteric lymph node (mesLN), and liver at time points indicated after *T. gondii* infection;  $N = 3$  independent experiments,  $n = 3$  to 4 mice per time point. (D) Lymphocytes were prepared from the siLP of naive or day-40 infected mice, stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin

and stained intracellularly for IFN- $\gamma$  and IL-17. Bar graph shows the percent (left) or the mean fluorescence intensity (right) of CD4<sup>+</sup> cells expressing IFN- $\gamma$  or IL-17. Shown is a representative example of three separate experiments;  $n = 4$  to 6 mice per experiment. (E) CD4<sup>+</sup> T cells were purified from the siLP by means of flow cytometry and cocultured with bone marrow–derived DCs preloaded with soluble *T. gondii* antigen (STAg) or activated with PMA/ionomycin for 3.5 hours. Stimulated cells were stained intracellularly for IFN- $\gamma$  and IL-17 and analyzed by means of flow cytometry. Shown is one of two experiments. Flow cytometry of CD4 cells is gated Live TCR $\beta$ <sup>+</sup> CD4<sup>+</sup> Foxp3<sup>−</sup>. Graphs show mean  $\pm$  SEM. \*\*\* $P < 0.001$ .

immune tolerance to commensal-derived antigens in the gastrointestinal (GI) tract is critical because activated CD4 T cells have been strongly associated with inflammatory bowel disease (IBD) (7, 8).

The GI tract is a common site of infection, and whether the immune system discriminates commensals from pathogens is unclear. In some instances, the proinflammatory properties of the microbiota directly contribute to the induction of immune responses and pathogenesis of mucosal infection (9–11). Whether acute GI infection also leads to priming of adaptive immune responses against commensals has not been addressed. To investigate the fate of immune responses to commensal bacteria after infection, we used *Toxoplasma gondii*. Upon oral infection, *T. gondii* expands systemically in the spleen and locally in the small intestine lamina propria (siLP), where it induces substantial immunopathology that is associated with a T helper 1 (T<sub>H</sub>1) immune response and a reduction in regulatory T cells (T<sub>reg</sub> cells) (Fig. 1A and fig. S1) (12). After day 9 of infection, the immune response largely eliminates the parasite from all tissues except the central nervous system and skeletal muscle, where *T. gondii* are encysted in an inactive state (Fig. 1A)

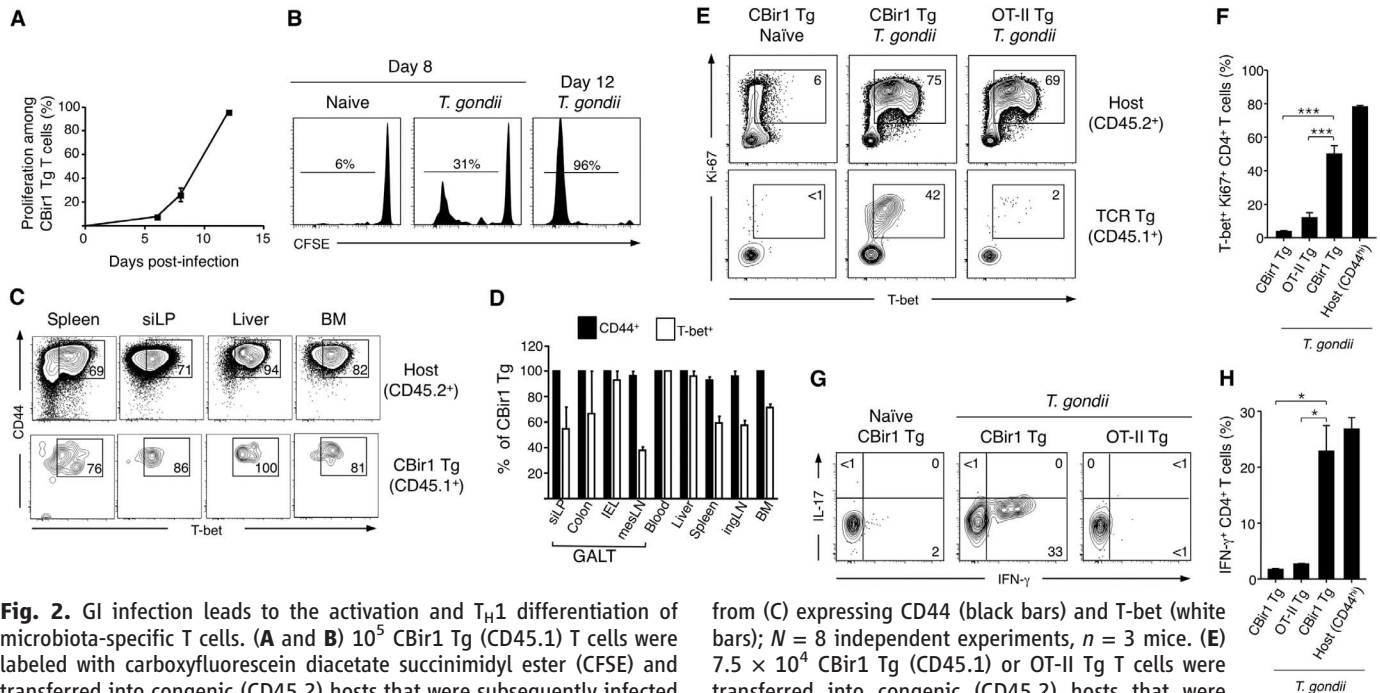
(13). Oral *T. gondii* infection also leads to an increased association between the commensal bacteria and the intestinal epithelium (Fig. 1B) (10). Moreover, bacteria escape the gut and translocate to the mesenteric lymph nodes, liver, and spleen (Fig. 1C). We postulated that immune ignorance of the microbiota may be lost during *T. gondii* infection as a result of bacterial translocation and impaired immune regulation.

Although the gut heals after clearance of *T. gondii*, the siLP harbored a significantly higher frequency of interferon- $\gamma$  (IFN- $\gamma$ ) CD4 T cells as compared with that of naïve mice, whereas the frequency of interleukin 17A (IL-17A)-expressing T cells remained unchanged (Fig. 1D). As expected, a fraction of these cells (~10% of CD4 T cells) were *T. gondii*-specific (Fig. 1E). However, CD4 T cells making IFN- $\gamma$  in response to nonspecific stimulation far outnumbered those responding to *T. gondii*-derived antigens (~45% of CD4 T cells), implying that a proportion of these cells may be specific to commensal bacteria (Fig. 1E).

To circumvent the high diversity of commensal antigens, we used a T cell receptor (TCR) transgenic mouse specific to a commensal-derived flagellin (CBir1 Tg), expressed by a subset of the

Clostridium XIVa cluster of bacteria (CBir1) (fig. S2) (14, 15). CBir1 is clinically relevant because antibodies to CBir1 flagellin are associated with Crohn's disease (16). Because of the segregation imposed by the mucosal firewall, splenic T cells from CBir1 Tg mice remain largely naïve (fig. S3) (15). Upon transfer into *T. gondii*-infected hosts, CBir1 Tg T cells proliferated extensively, whereas T cells that had been transferred into uninfected hosts remained undivided (Fig. 2, A and B). Thus, T cells specific to commensal-derived antigens proliferate during a heterologous GI infection.

We next addressed whether commensal-specific T cells would differentiate to become effector cells during GI infection. After *T. gondii* infection, CBir1 Tg T cells on WT or Rag<sup>-/-</sup> background differentiated toward a T<sub>H</sub>1 phenotype, as demonstrated by the expression of the canonical transcription factor T-bet (Fig. 2, C and D, and fig. S4). On the other hand, ovalbumin-specific OT-II TCR transgenic cells that had been transferred into *T. gondii*-infected mice remained naïve, and CBir1 Tg T cells did not respond to direct stimulation with *T. gondii* antigens, indicating that CBir1 Tg T cell responses rely on cognate antigen recognition (Fig. 2, E and F, and fig. S5).



**Fig. 2.** GI infection leads to the activation and T<sub>H</sub>1 differentiation of microbiota-specific T cells. (A and B) 10<sup>5</sup> CBir1 Tg (CD45.1) T cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and transferred into congenic (CD45.2) hosts that were subsequently infected orally with 15 cysts of *T. gondii*. At 6, 8, or 12 days after infection, splenocytes were isolated, and the dilution of CFSE on CBir1 Tg T cells (Live TCR $\beta$ <sup>+</sup> CD4<sup>+</sup> CD45.1<sup>+</sup>) was assessed by means of flow cytometry; *n* = 3 to 4 mice per time point. (C) 7.5 × 10<sup>4</sup> CBir1 Tg (CD45.1) T cells were transferred into congenic (CD45.2) hosts that were subsequently infected orally with 15 cysts of *T. gondii*. Eighteen days after infection, single-cell suspensions were prepared from the spleen, ingLN, mesLN, bone marrow, liver, peripheral blood, siLP, intraepithelial lymphocytes, and colon and stained intracellularly for flow cytometry. Contour plots show expression of CD44 and T-bet among CBir1 Tg (CD45.1) CD4 T cells (bottom row) and host CD4<sup>+</sup> T cells. (D) Bar graph showing the frequency of CBir1 Tg T cells

from (C) expressing CD44 (black bars) and T-bet (white bars); *N* = 8 independent experiments, *n* = 3 mice. (E) 7.5 × 10<sup>4</sup> CBir1 Tg (CD45.1) or OT-II Tg T cells were transferred into congenic (CD45.2) hosts that were subsequently infected orally with 15 cysts of *T. gondii* or left naïve. Eight days after infection, splenocytes were isolated and stained intracellularly for flow cytometry. Flow cytometric analysis of T-bet and Ki67 in CBir1 Tg or OT-II Tg (bottom row) and host CD44<sup>hi</sup> CD4 T cells (top row) is shown. Shown is a representative example of eight separate experiments. (F) Quantification of (E). (G) Splenocytes from (E) were stimulated with PMA/ionomycin, stained intracellularly for IFN- $\gamma$  and IL-17, and analyzed by means of flow cytometry. (H) Quantification of (G). Data are representative of three separate experiments. All flow cytometry plots in this figure are gated on Live TCR $\beta$ <sup>+</sup> CD4<sup>+</sup> Foxp3<sup>-</sup>. Graphs show mean ± SEM; \**P* < 0.05, \*\*\**P* < 0.001.



Activated commensal-specific T cells produced IFN- $\gamma$  in response to ex vivo stimulation and were observed in all tissues examined, indicating that anti-commensal T cell responses are systemic and functional (Fig. 2, C, D, G, and H; and fig. S6). Taken together, our data show that during GI infection CD4 T cell ignorance of commensal antigens is lost, and microbiota-specific T cells respond in a manner comparable with pathogen-specific T cells.

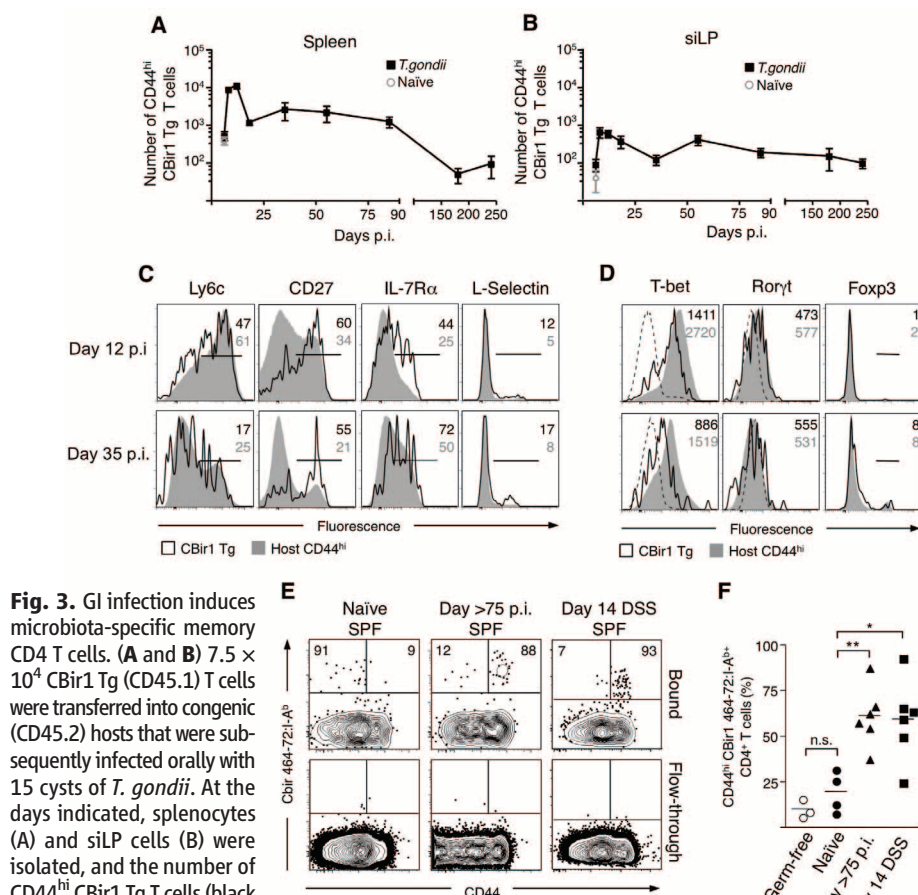
Under homeostatic conditions, the commensal microbiota can drive the differentiation of T<sub>reg</sub> and T<sub>H</sub>17 cells (11, 17–21). However, CBir1 Tg T cells did not induce the expression of IL-17A or the transcription factors Ror $\gamma$ t or Foxp3 during *T. gondii* infection (Figs. 2G and 3D), indicating

that microbiota-specific T cells are differentiating according to signals provided by the inflammatory milieu that are not typically present at steady state. Supporting this hypothesis, we found that CBir1 Tg T cells activated during chemical disruption of the GI tract by dextran sodium sulfate (DSS) did not up-regulate T-bet but instead expressed Ror $\gamma$ t (fig. S7).

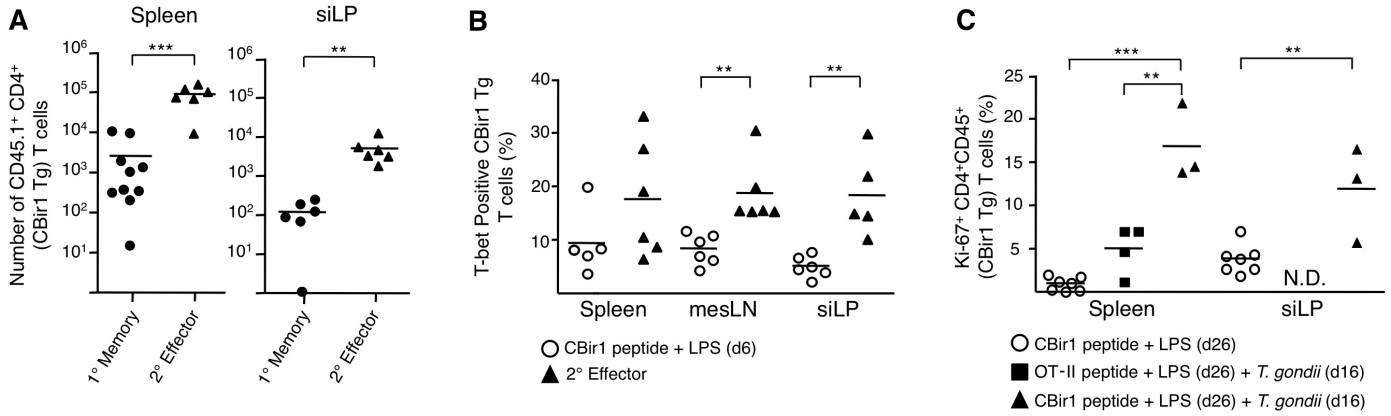
Immunological memory is a cardinal property of the adaptive immune system allowing for long-term protection against reinfection. During acute infections, T<sub>H</sub>1 T cell responses persist after the clearance of the pathogen but slowly decay over time (22, 23). Whether microbiota-specific effector T cells persist as memory cells is a critical question. Assessment of CBir1 Tg T cell response

revealed that the number of effector (CD44<sup>hi</sup>) CBir1 Tg T cells expanded ~10-fold from day 6 to the peak of the anti-microbiota response at day 12 after infection in both the spleen and siLP (Fig. 3, A and B). In accordance with what has been observed for pathogen-specific CD4 T cell responses, CBir1 Tg T cells contracted after day 12 of infection (Fig. 3, A and B) (22, 23). However, small populations of CD44<sup>hi</sup> CBir1 Tg T cells can be identified from both the spleen and lamina propria up to 240 days after infection, indicating that microbiota-specific cells generated in the context of heterologous GI infection have the potential for long-term survival (Fig. 3, A and B). T<sub>H</sub>1 CD4 T cells with increased potential to survive and form memory can be separated from terminally differentiated cells by the characteristic expression of CD27 and low expression of Ly6c and T-bet (23–25). Strikingly commensal-specific T cells largely differentiate to a phenotype consistent with long-lived T<sub>H</sub>1 memory T cells (Fig. 3, C and D). Critically, survival of memory CBir1 Tg cells was not the consequence of continuous antigen exposure because naïve CBir1 Tg T cells that had been transferred into mice 90 days after infection with *T. gondii* did not respond (fig. S8). In order to control for possible artifacts associated with the transfer of transgenic T cells, we examined the endogenous anti-CBir1 flagellin response via a major histocompatibility complex class II multimer (26, 27). In agreement with our T cell transfer studies, the majority of CBir1 multimer-positive cells in specific pathogen- and germ-free mice were naïve (CD44<sup>lo</sup>) (Fig. 3, E and F). In contrast, 75 days after *T. gondii* infection the majority of CBir1-specific cells displayed an activated (CD44<sup>hi</sup>) phenotype, indicating that the endogenous population of CBir1-specific cells is activated after GI infection and survives long-term as memory cells (Fig. 3, E and F, and fig. S9). In accordance with results obtained with transferred CBir1 Tg T cells, CD44<sup>hi</sup> CBir1 tetramer-specific cells expressed lower levels of Ly6c and increased amounts of CD27 than did *T. gondii*-specific cells from the same time point of infection (fig. S10). The majority of CBir1-specific T cells was also activated in mice treated with DSS, highlighting the importance of GI tract integrity in maintenance of CD4 T cell ignorance to commensal antigens (Fig. 3, E and F). Therefore, commensal-specific T cells activated during GI infection survive long-term and persist in extra-lymphoid tissue in a manner consistent with that of pathogen-specific memory cells.

A fundamental property of memory T cells is the ability to proliferate rapidly and elicit effector functions in response to secondary challenge. Indeed, CBir1 Tg T cells were able to rapidly make IFN- $\gamma$  and IL-2 in response to stimulation with specific peptide 75 days after infection (fig. S11). To test whether persisting commensal-specific T cells also maintain proliferative potential, we infected mice carrying CBir1 Tg T cells then re-challenged the mice with CBir1 peptide 35 days



**Fig. 3.** GI infection induces microbiota-specific memory CD4 T cells. (A and B)  $7.5 \times 10^4$  CBir1 Tg (CD45.1) T cells were transferred into congenic (CD45.2) hosts that were subsequently infected orally with 15 cysts of *T. gondii*. At the days indicated, splenocytes (A) and siLP cells (B) were isolated, and the number of CD44<sup>hi</sup> CBir1 Tg T cells (black squares) in each tissue was assessed by means of flow cytometry. Open gray circle shows the number of CD44<sup>hi</sup> CBir1 Tg T cells in naïve mice 8 days after transfer of CBir1 Tg T cells;  $N = 1$  to 8 independent experiments,  $n = 3$  to 15 mice per time point. (C) Flow cytometric analysis of surface marker phenotype of transferred CBir1 Tg T cells (CD45.1) (black line) and host CD44<sup>hi</sup> T cells (CD45.2) (gray shaded) at day 12 and day 35 after infection isolated from the spleen. (D) Expression of transcription factors in CBir1 Tg T cells (CD45.1) (black line) and host CD44<sup>hi</sup> T cells (CD45.2) (gray shaded) at day 12 and day 35 after infection isolated from the spleen. Histograms in (C) and (D) are gated on Live TCR $\beta^+$  CD4<sup>+</sup>; data shown is representative of three separate experiments. (E) Flow cytometric analysis of CBir1<sub>464-72</sub>:I-A<sup>b</sup> tetramer-binding populations from the total secondary lymphoid tissue in naïve, after-day-75 *T. gondii*-infected and DSS-treated mice. The top row depicts the population bound during tetramer-specific separation; the bottom row shows the nonbinding column flow-through to show specificity. Plots are gated on DAPI<sup>-</sup> NK1.1<sup>-</sup> F4/80<sup>-</sup> CD11c<sup>-</sup> CD11b<sup>-</sup> B220<sup>-</sup> CD3<sup>+</sup> CD4<sup>+</sup>. (F) Bar graph shows the percent CD44<sup>hi</sup> of CBir1<sub>464-72</sub>:I-A<sup>b</sup> tetramer-binding cells in germ-free (open circles), naïve (solid circles), infected (triangles), and DSS-treated (squares) mice. Data from (E) are representative of three experiments. Graphs show mean  $\pm$  SEM; \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig. 4.** Microbiota-specific memory T cell are functional and can be reactivated by GI infection. (A)  $7.5 \times 10^4$  CBir1 Tg (CD45.1) T cells were transferred into congenic (CD45.2) hosts that were subsequently infected orally with 15 cysts of *T. gondii*. Thirty-five days after infection with *T. gondii*, mice carrying CBir1 Tg T cells were injected with CBir1 peptide and LPS. Numbers are of CBir1 Tg T cells (CD45.1) in the spleen and siLP at day 35 (1° memory) or 6 days after stimulation with CBir peptide and LPS (2° effector). Shown is a composite of three experiments. (B) Either naïve hosts carrying  $\sim 10^4$  naïve CBir1 Tg T cells (open circles) or day 35 (1° memory) *T. gondii*-infected mice

(black triangles) were injected with CBir peptide and LPS as in (A). Six days after injection, splenocytes were isolated and stained intracellularly for T-bet. Data shown is representative of three experiments. (C) Naïve hosts carrying  $\sim 10^4$  naïve CBir1 Tg or OT-II Tg T cells were injected with peptide and LPS. Ten days after immunization, these mice were infected with 15 cysts *T. gondii*. Shown are percent Ki67<sup>+</sup> CBir1 Tg T cells isolated from the spleen and siLP from mice 16 days after infection (26 days post-immunization). Data shown are representative of three separate experiments. CBir1 Tg T cells are gated Live TCRβ<sup>+</sup> CD4<sup>+</sup> Foxp3<sup>-</sup> CD45.1<sup>+</sup>. Graphs show mean  $\pm$  SEM; \*\**P* < 0.01, \*\*\**P* < 0.001.

later. Before peptide challenge, CBir1 Tg T cells were uniformly activated (fig. S12). Memory CBir1 Tg T cells proliferated after antigen-specific challenge and accumulated in both the spleen and siLP (34- and 42-fold expansion, respectively) (Fig. 4A). In comparison with naïve CBir1 Tg T cells after activation with peptide and lipopolysaccharide (LPS), memory CBir1 Tg T cells maintained a significant level of expression of T-bet and had notably reduced expression of IL-7Rα (Fig. 4B and fig. S13). Therefore after recall, commensal-specific memory cells have a phenotype distinct from primary effectors and associated with their previous polarization. We next tested whether an established commensal-specific T cell population could be reactivated by GI infection. To address this point, we activated naïve CBir1 Tg T cells in vivo with peptide and LPS, waited for the primary immune response to subside, and then infected mice with *T. gondii*. Secondary infection induced proliferation of a fraction of CBir1 Tg T cells in both the siLP and spleen, as indicated by expression of Ki67 (Fig. 4C). Thus, activated commensal-specific T cells can proliferate in response to infectious rechallenge.

The GI tract represents a major site of exposure to pathogens, and our results propose that these pathogenic exposures lead to long-lived anti-commensal immunity (28, 29). In support of this hypothesis, healthy human serum contains antibodies specific to the intestinal microbiota (30). Because of the abundance of commensal antigens, a fraction of the memory CD4 T cell population induced in response to GI infection is likely composed of various commensal-specific clones that together may constitute a substantial population. Further, our results suggest that primary immune responses to GI infections occur in

the context of broader secondary responses against commensals. Several genes involved in sustaining the intestinal barrier and CD4 function have been associated with IBD, and in the context of such mutations, regulation of activated commensal-specific T cells could be jeopardized, leading to immunopathology (7). Indeed, GI barrier dysfunction and infection have been shown to synergize to induce IBD (31). Because bacteria colonize all pathogen entryways, such as the skin, lung, and GI tract, our findings raise the question of whether immunity and inflammation at barrier sites is generally controlled by responses to commensals.

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#### Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1220961/DC1  
Materials and Methods  
Figs. S1 to S13

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- **Senior Scientist/Research Associate** in animal models of autoimmune and inflammatory diseases
- **Senior Scientist** who is a **Biochemist/ Protein Conjugation Chemist** with experience in chemical conjugation of proteins (antibodies)
- **Senior Scientist/Research Associate** in animal models of cancer

Post Doctoral Fellowships in all areas are also available.

For more information on BIIR, please visit our  
website: [www.biir.org](http://www.biir.org)

Apply online at [www.baylorhealthcareers.com/science](http://www.baylorhealthcareers.com/science)  
or contact **James Smyda** at  
[James.Smyda@baylorhealth.edu](mailto:James.Smyda@baylorhealth.edu) or 972-291-4573.





## Institut Pasteur

### Head of Proteomics Core Facility

The Institut Pasteur opens a tenured Research Engineer position to recruit the head of its proteomics core facility. This facility is part of the Proteopole and is attached to a recently created Structural Mass Spectrometry and Proteomics Research Unit headed by Dr. Julia Chamot-Rooke.

The candidate will

- Manage an advanced proteomics group
- Develop a wide range of services based on robust procedures
- Oversee instrumentation
- Collaborate with the teams on the Pasteur campus and elsewhere
- Support and train in-house scientists
- Apply for grants for equipment
- Undertake technology development together with the Unit and other groups

Required qualifications, experience and skills

- PhD in mass spectrometry, analytical biochemistry or related areas
- Extensive practical experience in proteomics (ideally on a core facility)
- Experience in the field of quantitative and qualitative mass spectrometry including sample preparation, analytics, data processing and evaluation
- Excellent interpersonal, written and verbal communication skills
- Fluency in reading and speaking English and good level in French
- Successful interdisciplinary collaborations
- Experience in grant acquisition and project management

We offer

- The possibility to work on interesting scientific projects in an international and exciting environment
- Tenured position
- State-of-the-art instrumentation

Deadline for applications: **30 November 2012**

Applications should be sent to [proteoposition@pasteur.fr](mailto:proteoposition@pasteur.fr) and should comprise the following (in order) in a single pdf file:

1. A brief introductory letter.
2. A Curriculum Vitae and a full publication list.
3. A description of past and present activities, experience and skills (up to 8 pages with 1.5 spacing).

For more information please contact **Julia Chamot-Rooke** ([julia.chamot-rooke@pasteur.fr](mailto:julia.chamot-rooke@pasteur.fr))



## CHILDREN'S MEDICAL CENTER RESEARCH INSTITUTE AT UT SOUTHWESTERN

### Faculty Position in Stem Cell Biology

**The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a tenure-track faculty position in the area of stem cell biology.** Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct an independently-funded research program exploring any aspect of stem cell biology.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to [CRIApplicants@utsouthwestern.edu](mailto:CRIApplicants@utsouthwestern.edu).

*UT Southwestern is an Equal Opportunity/  
Affirmative Action Employer.*



## Institut Pasteur

### RESEARCH GROUPS IN NEUROSCIENCE

The Institut Pasteur in Paris announces an international call for outstanding candidates for tenure-track positions at both junior and senior level, in order to establish independent research groups within the Department of Neuroscience. The candidates will develop research programs on fundamental and clinical-related aspects of neuroscience.

Applications for the position of group leader will be accepted before **31 December 2012**, and will be evaluated on the basis of scientific excellence and complementarities with existing research goals of the department.

For junior positions, an attractive start-up package will include the salary for the group leader, a three-year postdoctoral position, a technician, part-time secretarial assistance, a substantial contribution to running costs and equipment, and access to on-campus facilities including state-of-the-art technology platforms. For senior positions, the start-up package will be discussed.

The application should comprise the following (in order) in a single pdf file:

1. A brief introductory letter.
2. A Curriculum Vitae and a full publication list.
3. A description of past and present research activities (4-5 pages with 1.5 spacing).
4. The proposed research project (8-10 pages with 1.5 spacing).
5. The names of 3 scientists from whom letters of recommendation can be sought.

Applications and requests for information should be addressed to [neuropositions@pasteur.fr](mailto:neuropositions@pasteur.fr). Further information on Institute and on-campus facilities can be found on the web site <http://www.pasteur.fr>. Short-listed candidates will be invited for interview in early 2013 and decisions will be announced by **June 2013**.



## Institut Pasteur

### RESEARCH GROUPS IN PARASITOLOGY AND MYCOLOGY

The Institut Pasteur in Paris announces an international call for outstanding candidates to establish independent research groups at all levels in the Department of Parasitology and Mycology and to develop research programs on pathogenic eukaryotic microorganisms (parasites or fungi) and/or their insect vectors.

Applications for the position of group leader will be accepted before **31 December 2012**, and will be evaluated on the basis of scientific excellence and complementarity with existing research goals of the department.

An attractive start-up package will include the salary for the group leader, a three-year postdoctoral position, a technician, part-time secretarial assistance, a substantial contribution to running costs and equipment, and access to on-campus facilities including state-of-the-art technology platforms.

The application should comprise the following (in order) in a single pdf file:

1. A brief introductory letter.
2. A Curriculum Vitae and a full publication list.
3. A description of past and present research activities (4-5 pages with 1.5 spacing).
4. The proposed research project (8-10 pages with 1.5 spacing).
5. The names of 3 scientists from whom letters of recommendation can be sought, together with the names of scientists with a potential conflict of interest from whom evaluations should not be requested.

Applications and requests for information should be addressed to [paramycopositions@pasteur.fr](mailto:paramycopositions@pasteur.fr). Further information on Institute and on-campus facilities can be found on the web site <http://www.pasteur.fr>. Short-listed candidates will be invited for interview in early 2013 and decisions will be announced by **June 2013**.



Institut Pasteur



### Call for Candidates to head 5-year groups in the Institut Pasteur in Microbiology and Virology

To strengthen its basic research in microbiology, and to improve its preparedness to respond to newly emerging infectious agents, Institut Pasteur calls for applications by outstanding young microbiologists to head Young Investigators Groups called Five Year Groups (G5).

The nominees will join the Laboratory of Excellence (LabEx) programme called, "Integrative Biology of Emerging Infectious Diseases" (IBEID)(<http://www.pasteur.fr/labex/ibeid>). They will benefit from the cutting edge interdisciplinary environment provided by the Institut Pasteur in Paris and by the LabEx.

Examples of subject priorities in microbiology include molecular mechanisms of resistance to antibiotic, regulatory mechanisms of gene expression in bacteria, and mechanisms of homeostasis and communication in complex microbial communities. In virology, priority will be given to projects addressing viruses with strong potential for emergence, such as arbovirus.

However, all aspects of microbiology and virology will be considered, and outstanding candidates with an ambitious and original basic research project are strongly encouraged to apply.

The deadline for applications is **December 15th, 2012**. Short-listed candidates will be called for interview in **January 2013** and decisions will be announced by **March 30**.

**General conditions and applications.** Candidates must have defended their PhD thesis after November 30, 2004. Successful candidates will be appointed as head of a group of up to 6 people for a period of 5 years. The budget includes the salary for the group leader (if necessary), a three-year postdoctoral position, a technician, part-time secretarial assistance, basic laboratory equipment, a substantial contribution to running costs and essential large equipment, and access to on-campus facilities including state-of-the-art technology platforms.

Candidates should send their formal applications by E-mail to the Director of Scientific Evaluation, **Prof. Alain Israël**, at the Institut Pasteur (**25, rue du Dr. Roux, 75724 Paris, France; [g5ibeid@pasteur.fr](mailto:g5ibeid@pasteur.fr)**).

The Application shall comprise the following (in order) in a single pdf file:

1. A brief introductory letter (candidates are encouraged to contact the coordinators of the LabEx, **Pascale Cossart** ([pascale.cossart@pasteur.fr](mailto:pascale.cossart@pasteur.fr)) or **Philippe Sansonetti** ([philippe.sansonetti@pasteur.fr](mailto:philippe.sansonetti@pasteur.fr)))
2. A Curriculum Vitae and a full publication list.
3. A description of past and present research activities (up to 5 pages with 1.5 spacing).
4. The proposed research project (up to 10 pages with 1.5 spacing) and how it would fit in the defined topic.
5. The names of 3 scientists from whom letters of recommendation can be sought, together with the names of scientists with a potential conflict of interest from whom evaluations should not be requested.



## Faculty Position for Basic or Clinician Scientist as Assistant, Associate or Full Professor

### Department of Ophthalmology

The UCSF Department of Ophthalmology, in conjunction with the UCSF Biomedical Sciences Program and the Neuroscience Program, is initiating a search for research faculty at the Assistant, Associate, or Full Professor rank. Areas of interest include, but are not limited to, immunology, cell & molecular biology, genetics, neuroscience, and regenerative medicine. Candidates pursuing basic and disease-oriented translational research with relevance to vision-threatening conditions will be expected to develop a substantial research program capable of obtaining independent, external funding and to capitalize on the collaborative research environment at UCSF. Responsibilities include participation in graduate student, medical student, resident, and fellow teaching as appropriate.

**Requirements:** PhD and/ or MD, at least 2 years of postdoctoral experience, and evidence of high quality independent research. Candidates whose research program will forge strong ties to basic science departments on campus are specifically encouraged to apply. Housing assistance is available.

Interested applicants should send a single pdf file with a cover letter, CV, research plan (2 pages max.), and at least three letters of recommendation (or names of references) by December 15th, 2012 to:

**[job@vision.ucsf.edu](mailto:job@vision.ucsf.edu)**

Address letters to Jacques Duncan, MD, and David Sretavan, MD, PhD, Search Committee Co-Chairs.

*UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence.*

*UCSF is an affirmative action/equal opportunity employer. The University undertakes affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for covered veterans. All qualified applicants are encouraged to apply, including minorities and women.*

SCHOOL OF PHARMACY

SCHOOL OF NURSING

SCHOOL OF DENTISTRY

SCHOOL OF PHARMACY

SCHOOL OF NURSING

SCHOOL OF DENTISTRY

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SCHOOL OF PHARMACY

SCHOOL OF MEDICINE SCHOOL OF NURSING





Memorial Sloan-Kettering  
Cancer Center

*The Best Cancer Care. Anywhere.*

## TENURE TRACK FACULTY POSITION Cancer Pharmacology or Drug Discovery

Sloan-Kettering Institute is seeking an innovative individual who wishes to address problems of relevance to cancer drug discovery and development for a tenure-track position at the Assistant or Associate Member level with strong research accomplishments in biology, biochemistry or pharmacology. Faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Graduate School of Medical Sciences of Cornell University, as well as the Tri-Institutional MD/PhD Training Program and Tri-Institutional Training Program in Chemical Biology.

MSKCC offers a unique and exciting research environment with programs in Immunology, Pharmacology, Chemistry, Molecular Biology, Computational Biology, Genetics, Cell Biology, Developmental Biology, Cellular Biochemistry and Structural Biology. The presence of world-renowned clinical programs in cancer research, treatment and prevention offers unique opportunities for creative collaboration.

Applicants should have a PhD and/or MD degree, postdoctoral experience, and dedication to important problems at the interface of biology, biochemistry or chemistry as they relate to cancer pharmacology.

The deadline for applications is **November 1, 2012**. Interested candidates should visit <http://facultysearch.ski.edu> to access the on-line faculty application. Please visit the site as soon as possible, as it contains important information on the required application materials, including deadlines for submission of letters of reference. Inquiries may be sent to **Marie Aiello** at [aiello@mskcc.org](mailto:aiello@mskcc.org) or to **Dr. David Scheinberg**, Chairman, Molecular Pharmacology and Chemistry Program at [scheinbd@mskcc.org](mailto:scheinbd@mskcc.org).

**facultysearch.ski.edu**

MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.



CHILDREN'S MEDICAL CENTER  
RESEARCH INSTITUTE  
AT UT SOUTHWESTERN

### Faculty Position in Metabolism Research

The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a **tenure-track faculty position in the area of metabolism and disease**. Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct independently-funded research programs. Areas of specific interest include analysis of metabolism at the cellular level, including metabolomics, metabolic flux analysis, metabolic imaging and mitochondrial biology. In addition to analytical equipment dedicated to the investigator's studies, CRI members will also have access to a metabolomics core with triple-quadrupole mass spectrometry and gas chromatography/mass spectrometry. NMR spectroscopy, <sup>13</sup>C dynamic nuclear polarization, a human 7-Tesla MRI and a state-of-the-art mouse metabolic phenotyping facility are also available on campus to provide an unparalleled breadth of metabolic analysis.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to [CRIApplicants@utsouthwestern.edu](mailto:CRIApplicants@utsouthwestern.edu).

*UT Southwestern is an Equal Opportunity/Affirmative Action Employer.*



## FACULTY POSITION IN BIOCHEMISTRY

Applications for a tenure-line faculty position (preferably at the Assistant or Associate Professor level) applying NMR to biological problems are invited by the Department of Biochemistry and School of Medicine at the University of Missouri in Columbia. We encourage individuals applying NMR to biological questions in many areas including, but not limited to metabolomics of disease, membrane proteins, intrinsically disordered proteins, protein complexes, solids and tissues, and biophysical enzymology. The Bruker 800, 500, and Varian 600 MHz spectrometers available on campus are each equipped with a cryogenic probe. Also available on campus are Bruker Avance III 7T micro-MRI and Siemens Trio 3T MRI systems capable of *in vivo* spectroscopy. The University is noted for interdisciplinary research in life sciences, including biophysical areas. Position qualifications include a Ph.D. or M.D. in biochemistry, biophysics or a related field, and postdoctoral experience. The successful applicant will develop or continue an outstanding, independently funded research program and contribute to departmental teaching activities.

Submit the following information to <http://hrs.missouri.edu/find-a-job/academic/index.php>: (1) a cover letter; (2) curriculum vitae; (3) a 4-5 page description of current and planned research; and (4) the names and contact information of three references. Please use the Job Opening Number **8537**. Application review will begin **October 22<sup>nd</sup>, 2012**.

*MU is an EEO/AA/ADA Employer, and encourages applications from women and minorities. For ADA accommodations, contact our Human Resource Services (573) 882-7976.*



DANA-FARBER  
CANCER INSTITUTE

## Instructor, Assistant or Associate Professor of Medicine Gastrointestinal Cancer

The Dana-Farber Cancer Institute, Brigham and Women's Hospital and the Department of Medicine, Harvard Medical School, invite applications for a full-time appointment at the Instructor, Assistant or Associate Professor level. This individual will develop an independent, disease-based laboratory research program focused on Gastrointestinal Cancers housed in the Division of Molecular and Cellular Oncology and will interface with the clinical and translational research program within the Division of Gastrointestinal Oncology. The successful candidate should be committed to innovative scientific research and, working with the Division of Gastrointestinal Oncology, advancing these discoveries toward novel therapeutic strategies. Applicants must have an MD and/or PhD and a proven track record of outstanding laboratory research.

The candidate will work principally at the Dana-Farber Cancer Institute, an NCI-designated Comprehensive Cancer Center, and at the Brigham and Women's Hospital. The academic appointment will be at the Instructor, Assistant or Associate Professor level. Salary and benefits will be competitive with other institutions and commensurate with experience (or accomplishments, or qualifications).

**Interested candidates must submit a curriculum vitae, a research plan and three letters of reference to: William Hahn, MD, PhD, Chief, Division of Molecular and Cellular Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Please send submissions via email to: [MCO\\_SEARCH@dfci.harvard.edu](mailto:MCO_SEARCH@dfci.harvard.edu).**



HARVARD  
MEDICAL SCHOOL



BRIGHAM AND  
WOMEN'S HOSPITAL

Dana-Farber Cancer Institute/Brigham and Women's Hospital/Harvard Medical School are Equal Opportunity/Affirmative Action Employers actively committed to increasing the diversity of our faculty; people with disabilities, veterans, women and members of underrepresented minority groups are therefore strongly encouraged to apply.

Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. The University has a comprehensive range of study programmes and research disciplines spread across 10 faculties and about 100 sub-divisions of studies and learning. There are over 23,400 undergraduate and postgraduate students coming from 50 countries, and more than 1,800 members of academic and academic-related staff, many of whom are internationally renowned.

### Post-doctoral Fellowships and Research Assistant Professorships

Applications are invited for a number of positions as Post-doctoral Fellow (PDF) and Research Assistant Professor (RAP), at the University of Hong Kong, on or before July 31, 2013. Appointments will be made for a period of 2 to 3 years.

PDF and RAP posts are created specifically to bring new impetus and vigour to the University's research enterprise. Positions are available from time to time to meet the strategic research needs identified by the University. Positions are available in the following Faculties/Departments/Schools/Centres:

- School of Chinese
- School of Modern Languages and Cultures
- School of Business
- Faculty of Dentistry
- Faculty of Education
- Computer Science
- Electrical and Electronic Engineering
- Industrial and Manufacturing Systems Engineering
- Mechanical Engineering
- Professional Legal Education
- Centre for Cancer Research
- Research Centre of Heart, Brain, Hormone and Healthy Aging
- Centre of Influenza Research
- Medicine
- Pharmacology and Pharmacy
- Physiology
- Public Health Research Centre
- Centre for Reproduction, Development and Growth
- Surgery
- Chemistry
- School of Biological Sciences
- Psychology
- The State Key Laboratory of Brain and Cognitive Sciences

#### Post-doctoral Fellows

PDFs are expected to devote full-time to research. Applicants should be doctoral degree holders having undertaken original research that has contributed to the body of knowledge. A highly competitive salary commensurate with qualifications and experience will be offered. Annual leave and medical benefits will also be available.

#### Research Assistant Professors

The main focus of an RAP's duty is research. RAPs can however be assigned some teaching duties, up to 50% of the normal teaching load. Applicants should be research active and have a proven publication record. A highly competitive salary commensurate with qualifications and experience will be offered, with a contract-end gratuity and University contribution to a retirement benefits scheme (totalling up to 15% of basic salary). Annual leave, and medical/dental benefits will also be offered.

#### Procedures

Prospective applicants are invited to visit our webpage at <http://jobs.hku.hk/> to view the list of the Faculties/Departments/Schools/Centres and their research areas for which PDF/RAP positions are currently available. Before preparing an application, they should contact the Head of the appropriate academic unit to ascertain that their research expertise matches the research area for which a vacant PDF/RAP post is available.

Applicants must submit a completed University application form, which should clearly state **which position they are applying for**; and in which academic discipline. They should also provide further information such as details of their research experience, publications, research proposals, etc.

Application forms (341/1111) can be obtained at <http://www.hku.hk/apptunit/form-ext.doc>. Further particulars can be obtained at <http://jobs.hku.hk/>. **Closes October 20, 2012.** The University thanks applicants for their interest, but advises that only shortlisted applicants will be notified of the application result.

The University is an equal opportunity employer and is committed to a No-Smoking Policy



## Positions in the Faculty of Health Sciences

As a flagship higher education institution in Macao, the University of Macau is progressing rapidly with strategic initiatives including construction of a new campus with 80+ buildings and more than USD16B investment, development of the largest Residential College system in Asia, and establishment of a new Faculty of Health Sciences.

### The Faculty of Health Sciences

The new Faculty of Health Sciences aims at providing excellent education programs, performing outstanding research, and delivering dedicated services to the community. In particular, the new Faculty plans to set up a number of research centers dedicated, but not limited to research areas in aging, biotechnology, cancer, development biology, infectious diseases, metabolism, molecular medicine, neurodegenerative disorders, preventive medicine, public health, and systems biology, etc.

The University of Macau now invites applications in these areas in the Faculty of Health Sciences ranging from Chair/Distinguished/Full/Associate/Assistant Professors, Directors/Managers for Core Facilities, Sub-team Leaders, Administration Officers/Senior Administration Officers, Instructors/Senior Instructors, Postdoctoral Fellows to Biologists/Technicians. Subject to negotiation, incumbents may also be concurrently appointed to other leadership positions in the Faculty, such as Associate Dean, Center Director, depending on the qualification of candidates and need of the Faculty.

### Position and Remuneration

Remuneration and appointment rank offered will be competitive and commensurate with the successful applicants' academic qualification and professional experience. The current local maximum income tax rate is 12%, while after various discretionary exemptions the effective income tax rate has been around 5% - 7%. English is the University's working language.

### Application Procedure

Application should include a cover letter indicating positions interested, a brief statement of research interest (required for independent professorship positions), and current curriculum vitae in English. Review of applications will commence on October 5, 2012 and continue until the positions are filled. Applicants should visit the University website <http://www.umac.mo/vacancy> for more details, and apply **online** at <https://isw.umac.mo/recruitment> (Ref. No.: FHS/AR/09/2012). For inquiry, please contact

#### Human Resources Office

University of Macau, Av. Padre Tomás Pereira, Taipa, Macau

Website: <https://isw.umac.mo/recruitment>; Email: [vacancy@umac.mo](mailto:vacancy@umac.mo)

Tel: +853 8397 8593 or +853 8397 8592; Fax: +853 8397 8694

The effective position and salary index are subject to the Personnel Statute of the University of Macau in force. The University of Macau reserves the right not to appoint any candidate in any category.

Applicants with less qualification and experience can be offered lower positions under special circumstances.

\*\*\*Personal data provided by applicants will be kept confidential and used for recruitment purpose only\*\*\*

University of Macau –  
An ideal place to pursue your career



## Faculty Position in Bioenergy-Related Synthetic Biology at Arizona State University – Tempe, AZ

The School of Life Sciences at Arizona State University invites applications for a tenure-track faculty position at the level of Assistant Professor in the area of Synthetic Biology / Metabolic Engineering related to Bioenergy. We encourage applications from outstanding candidates who employ an integrated and innovative approach to generate new or recombined metabolism in organisms that may lead to, for example, useful products or fuels made from sunlight, CO<sub>2</sub>, and/or other readily available compounds. Candidates employing metabolite and metabolic flux analysis, -omics approaches, bioinformatics, genetic engineering, and/or related approaches are preferred. Candidates with clear potential to develop a strong, extramurally funded, independent research program, teach at the undergraduate and graduate levels, and mentor graduate and undergraduate students and postdocs will be given preference. A competitive start-up package and teaching load compatible with high research productivity will be provided.

Arizona State University has a vibrant, interdisciplinary research and education community in the area of solar energy conversion and utilization. The successful candidate will become part of this transdisciplinary community that is organized at several levels, including the Bioenergy and Photosynthesis Center (<http://bioenergy.asu.edu/>) and LightWorks ([asulightworks.com](http://asulightworks.com)).

Candidates must have a Ph.D. (or equivalent) in biology, biochemistry, engineering, or a related field. A record of accomplishment that illustrates strong preparedness for establishing an excellent, independent research program in the area of the search is desired, along with teaching experience. To apply, send a cover letter, your curriculum vitae, three representative publications, contact information for at least three references, and separate statements of future research plans and teaching philosophy/interests in a single pdf file to [solsfacultysearch4@asu.edu](mailto:solsfacultysearch4@asu.edu). The initial closing date for receipt of applications is **October 28, 2012**, applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on the School of Life Sciences, please visit <http://sols.asu.edu>.

*Arizona State University is an Equal Opportunity/Affirmative Action Employer committed to excellence through diversity. We especially encourage women and minorities to apply.*

## Foundation Fellow Positions in Biochemistry and Molecular Cell Biology



The College of Science and Mathematics at Kennesaw State University invites applications for two senior Foundation Fellow positions: Associate Professor of Biochemistry in the Department of Chemistry and Biochemistry, and Associate Professor of Molecular Cell Biology in the Department of Biology and Physics. Highly qualified candidates will be considered for hiring at the level of Professor. Requirements are an earned doctorate in an appropriate discipline and a demonstrated record of significant research activity and external funding. Successful candidates will assume leadership roles in our research community and participate in our MS and undergraduate programs.

For a complete description of positions, go to <http://www.kennesaw.edu/facultypositions/>. To guarantee consideration, application materials should be received by October 26, 2011. Submit a letter describing qualifications for the position, a statement of teaching philosophy, a statement of research interests, a current curriculum vitae, graduate transcripts, and the names, addresses, telephone numbers, and e-mail addresses of three references to the appropriate search committee chair as directed in the full description of positions.

*Kennesaw State University is an affirmative action/equal opportunity employer and educator. Georgia is an Open Records State.*



## Neuroscience Faculty Recruitment

The Department of Neuroscience at Columbia University is currently recruiting faculty in the neurosciences, with a primary emphasis on three research areas: (1) cognitive and/or motor processes in awake, nonhuman primates; (2) linking neural circuitry and behavior in genetically tractable mammalian systems; and (3) molecular, cellular, and/or developmental neuroscience. In a cover letter, please direct your application to one of these three research areas. In exceptional circumstances, we will also consider applications in areas more distantly related to these three primary research themes. We encourage applications at all levels of seniority, from assistant to full professor.

Columbia University has an exceptionally strong and broad program in the neurosciences and aims to enhance interactions between basic and clinical research and to link the neurosciences with a wide range of other disciplines within the University. New faculty will be affiliated with the Department of Neuroscience, with the Doctoral Program in Neurobiology and Behavior, and with a newly established Mind Brain Behavior Institute. In addition, there are numerous opportunities for interaction with other scientific departments and programs at the Medical Center and Morningside Heights campuses.

The application deadline is November 30, 2012. Please submit applications online at <https://academicjobs.columbia.edu/applicants/Central?quickFind=56746> and include a cover letter, curriculum vitae, and a statement of research interests. In addition, please arrange for three references to submit letters of recommendation.

*Columbia University takes affirmative action to ensure equal opportunity.*

## Distinguished Scientific Research Opportunity Early Career Faculty Position

The Genomics Division at Lawrence Berkeley National Laboratory invites applications from early career scientists for a Divisional Fellow position at the DOE Joint Genome Institute (JGI, [www.jgi.doe.gov](http://www.jgi.doe.gov)). This position is equivalent to a tenure-track faculty position at a university, and is appropriate for scientists with a Ph.D. or M.D. degree who have demonstrated outstanding promise and creative ability during their post-doctoral or equivalent training. Divisional Fellows are appointed to a five-year term and are considered for accelerated promotion to Senior Scientist status at the end of this term. We are specifically seeking individuals to direct a research program that applies cutting-edge methods in **functional genomics, synthetic biology** and/or **genome informatics** in the areas of **plant, fungal, microbial, and metagenomic science**.

The JGI is a large-scale genome sciences facility with an emphasis on topics related to energy and environmental issues. The selected individual is expected to develop a strong independent research program. In addition they will have the opportunity to participate in large multidisciplinary research programs in collaboration with scientists throughout the JGI, LBNL, and the neighboring University of California, Berkeley campus.

For a detailed position description and instructions regarding how to apply, please visit [www.lbl.gov](http://www.lbl.gov), access the careers page and reference job number 75074.

Or, submit CV, summary of research interests, and references to Eddy Rubin, JGI Director  
c/o recruiter Bill Cannan: [WRCannan@lbl.gov](mailto:WRCannan@lbl.gov). AA/EOO





## Faculty Position in Infectious Disease, Immunity and/or Inflammation

The Virginia Tech Carilion Research Institute (VTCRI) in Roanoke, Virginia (<http://research.vtc.vt.edu/>) is recruiting a faculty member who works in the area of infectious disease, immunity and/or inflammation. The position may be filled at the tenured, tenure track or non-tenure track level as an Assistant, Associate or full Professor, as appropriate. The position is a 12 month appointment with primary emphasis on research. The successful candidate will have a Ph.D., M.D. or M.D./Ph.D. Established investigators with strong innovative research programs and extramural funding are encouraged to apply although promising junior candidates will be considered. Start-up packages, facilities and support are highly competitive. The successful candidate will interact with the molecular virology, cancer biology, developmental molecular genetics, and neurobiology research teams at the VTCRI as well as with an accomplished group of infectious disease, immunity and inflammation research teams in Biology, Biomedical Engineering and Sciences, and Veterinary Medicine at Virginia Tech.

The VTCRI opened in the summer of 2010 and currently has 20 faculty research team leaders in structural biology, molecular and neurobiology, molecular virology, tumor biology and genetics, cardiac and regenerative medicine. The Institute has state-of-the-art facilities in molecular biology, cell culture, optical imaging, high field cryo electron microscopy, magnetic resonance imaging, electrophysiology, computational and high capacity data analysis/storage and a new vivarium, as well as access to patient populations and samples with our colleagues in the infectious disease program at Carilion Clinic. During this period of major growth of the new institute, we are especially interested in colleagues who enjoy a highly collaborative environment and interacting with investigators from their own, as well as other disciplines including those working at molecular, cellular, systems and computational levels with animal models of human disease and/or humans. Investigators using molecular genetic, structural biology, microbiome and/or computational approaches to study the mechanisms and/or pathogenesis of human disease or animal models of infectious disease, the innate or adaptive immune response and/or inflammatory processes in response to infection are encouraged to apply.

The VTCRI is immediately adjacent to the Carilion clinic and hospital and the new VTC School of Medicine where all medical students carry out four year research projects. The Institute has strong collaborative ties with the Virginia Bioinformatics Institute (VBI) and the School of Biomedical Engineering and Sciences (SBES) at Virginia Tech (VT), as well as with the VT Departments of Biological Sciences, Biochemistry, Physics, Psychology and the College of Veterinary Medicine. The research institute and medical school are located in the picturesque Roanoke Valley midway between Washington, DC and Charlotte, NC.

Interested and competitive candidates should send a cover letter, their full CV, statement of research plans, and the names, full addresses and email addresses of three references to the attention of: **IDII faculty position-VTCRI at secastle@vtc.vt.edu** before November 1, 2012, as well as posting these materials on the Virginia Tech jobsite at <https://listings.jobs.vt.edu/applicants/Central?quickFind=196043>. The three support letters should be addressed to **Michael J. Friedlander, Ph.D., Executive Director, VTCRI** and sent by email directly from the referees to Ms. Sarah Castle at **secastle@vtc.vt.edu** indicating IDII Position.

*Virginia Tech is an Equal Opportunity/Affirmative Action Institution.*



CHILDREN'S MEDICAL CENTER  
**RESEARCH INSTITUTE**  
AT UT SOUTHWESTERN

### Faculty Position in Cancer Biology

The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a **tenure-track faculty position in the area of cancer biology**. Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct an independently-funded research program exploring any aspect of cancer biology.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to **CRIApplicants@utsouthwestern.edu**.

*UT Southwestern is an Equal Opportunity/  
Affirmative Action Employer.*

EBERHARD KARLS  
**UNIVERSITÄT  
TÜBINGEN**



WERNER REICHARDT CENTRE  
FOR INTEGRATIVE NEUROSCIENCE

### Junior Research Group for Neuroscientists at the Werner Reichardt Centre for Integrative Neuroscience (CIN) in Tübingen

The **Werner Reichardt Centre for Integrative Neuroscience (CIN)** is an interdisciplinary institution at the **Eberhard Karls University Tübingen** funded by the German Excellence Initiative program. The CIN strives to deepen our understanding of how the brain generates function and how brain diseases impair functions. It tries to make use of newly acquired insights to help people with brain disorders and to launch new mind- and brain-inspired applications in many areas of engineering and computer science. Its scientific program is guided by the conviction that progress in the understanding of brain function can only be achieved by an integrative approach spanning multiple levels of organization and pooling the knowledge of researchers from many different fields.

In order to strengthen specific research areas, the CIN offers three more junior group leader (JRG) positions with tenure track option for up-and-coming young scientists with a promising track record, working in **Cognitive Neuroscience, Systems Neuroscience or Neurotechnology**. The CIN strives to increase the number of female scientists. Therefore **qualified female candidates are explicitly encouraged to apply**. **Submission deadline is Oct. 15<sup>th</sup>.**

#### Framework

The intended duration of the position is for 5 years with evaluations by external experts at regular intervals. In the event of positive evaluations after 3 years, the JRG will obtain a tenure track option, which may ultimately lead to a professorship at the University of Tübingen. Start-up funds as well as substantial funding for personnel and running costs will be available, but will depend on the applicant's qualifications and prior experience. Appointees will be full members of and active participants in the CIN, which will also provide laboratory and/or office space. The JRG leader will be provided with opportunities to contribute to research oriented training within the framework of the CIN Graduate Training Centre and the faculties involved in the CIN will provide opportunities for the German habilitation according to established rules, if desired. According to German law, severely disabled persons with equal occupational aptitude will be given preferential consideration.

#### Application

Applicants should submit a curriculum vitae, pdf files of up to 5 key publications, a statement of research achievements and future directions (not to exceed 3 pages) as well as the names and addresses of at least three referees. All documents should be submitted electronically to the Chairman of the Werner Reichardt Centre for Integrative Neuroscience Tübingen, Prof. Dr. Peter Thier, at [cin@uni-tuebingen.de](mailto:cin@uni-tuebingen.de). For further information on the CIN see: <http://www.cin.uni-tuebingen.de/>. **Submission deadline is Oct. 15<sup>th</sup>.**





## School of Freshwater Sciences

### Faculty Position: Fisheries & Aquaculture (Assistant/Associate Professor/Professor)

The University of Wisconsin-Milwaukee (UWM) School of Freshwater Sciences (SFS) invites applications for an open rank faculty position (Assistant/Associate Professor/Professor) who will teach and perform applied research to solve the problems of sustainable freshwater fisheries and aquaculture with an emphasis on urban settings. We seek individuals who also have strong hands on experience and skill in the culture, husbandry, nutrition, and health of fin fish. The SFS is developing a national center for urban aquaculture and the successful candidate will help lead that effort and participate in cooperative programs with the USDA/ARS and other government agencies, the aquaculture industry, planners, and economists.

Applicants must hold a PhD or equivalent in aquatic science, biology, aquaculture, or a closely related field. Research experience in areas relevant to the position is highly desirable. The successful candidate is expected to develop vigorous, integrative, collaborative, extramurally funded research programs and apply tools and innovations of modern biology (e.g. genomics, proteomics, metabolomics, and bioinformatics), physical sciences, and engineering to help solve problems facing freshwater fisheries and aquaculture.

The School of Freshwater Sciences expands a tradition of freshwater studies at UWM that has been carried out at the Great Lakes Research Facility since 1966. Research and education is integrated across four essential themes: freshwater system dynamics; human and ecosystem health; freshwater technology; and freshwater economics, policy, and management. In pursuing these multidisciplinary themes, SFS works with a wide range of partners inside and outside the university. SFS is a graduate degree only program and faculty teach and advise students at the graduate level.

Complete information can be found at <http://www4.uwm.edu/freshwater>.

**On-Line Application Procedure:** Application materials include: a cover letter describing your interest in and qualifications for the position including the name and contact information of three references; a curriculum vitae; a brief research plan; a teaching statement; and examples of published work. The application materials should be submitted electronically at <https://jobs.uwm.edu/postings/10479>. Initial screening of applications will begin on **November 1, 2012** and will continue until the position is filled. Questions should be directed to **Dr. John Janssen, Search Committee Chair, School of Freshwater Sciences, 600 East Greenfield Avenue, Milwaukee, WI 53204** or [jjanssen@uwm.edu](mailto:jjanssen@uwm.edu). Under Wisconsin's open records law, requests for confidentiality will be honored, except that names and titles of all finalists must be disclosed upon request. UWM offers competitive salary and startup packages, commensurate with experience. Further information about UWM may be found at [www.uwm.edu](http://www.uwm.edu).

*UWM is an Equal Opportunity/ Affirmative Action Employer.*



### Postdoctoral Fellowships Available

The Lombardi Comprehensive Cancer Center (LCCC) at Georgetown University, a multidisciplinary NCI-designated cancer research center, is currently recruiting postdoctoral fellows into positions funded by an NCI training grant. The goal is to develop strong basic and translational scientists with an interest in cancer research. Successful applicants will choose a mentor from an interdisciplinary group of investigators who are committed to cancer research. Research programs include:

- The role of growth factor signal pathways
- The development of hormone and drug resistance
- The genetic and molecular mechanisms of malignant progression
- Invasion metastasis angiogenesis
- Stem cells in cancer
- Role of metabolism in cancer
- Development of novel immunological and anticancer therapies
- The etiology of cancer, biomarkers, and molecular epidemiology
- Bioinformatics and cancer

Go to <http://lombardi.georgetown.edu/education/TBIO/postdoc.html> for further information. Salary is competitive and commensurate with qualifications and experience. Applicants should send curriculum vitae, a short statement of research interests and career goals, and the names and addresses of three references to **Karen Shepherd** at [bivinsk@georgetown.edu](mailto:bivinsk@georgetown.edu).

**Minorities and women are strongly encouraged to apply. US citizenship or permanent residency is required.**

## Keck School of Medicine of USC

Department of Stem Cell Biology and Regenerative Medicine  
Keck School of Medicine  
University of Southern California  
Assistant Professorships In Regenerative Medicine and  
Cancer Stem Cells

The Department of Stem Cell Biology and Regenerative Medicine is recruiting candidates whose research focuses on understanding fundamental principles of regenerative processes and developing knowledge-based approaches to organ repair. The Department is housed within the newly created Eli and Edythe Broad CIRM Center for Regenerative Medicine and Stem Cell Research within the W. M. Keck School of Medicine at the University of Southern California. Significant resources are available to support all aspects of stem cell research within the building and adjacent centers. Excellent collaborative opportunities exist across the USC campuses. The Department is seeking scholars researching regenerative mechanisms of tissue repair, and in conjunction with the Norris Cancer Center, scholars investigating cancer stem cells. In addition to its research mission, all members will play an important role in the educational mission of this newly created Department. Generous start-up packages will be awarded to the successful candidates.

Online applications will be accepted for each search, please apply specifically to the search in Regenerative Medicine (<https://jobs.usc.edu/applicants/Central?quickFind=66422>) or Cancer Stem Cells (<https://jobs.usc.edu/applicants/Central?quickFind=66421>). Applications should include a letter of interest, curriculum vitae, brief 2-3 page outline of research past, present and future, and **four** letters of reference. The applicant is responsible for ensuring the completed application is received before **November 21<sup>st</sup>, 2012**.

*Women and individuals belonging to minority groups are particularly encouraged to apply. The University of Southern California is an Equal Opportunity Affirmative Action Employer.*



## NORTHWESTERN UNIVERSITY

### Neurobiology Assistant Professor

The Department of Neurobiology, in the Weinberg College of Arts and Sciences, seeks to recruit a new tenure-track faculty member at the level of Assistant Professor. Applicants holding a Ph.D. and/or M.D. degree and demonstrating an outstanding record of scientific achievement will be considered. We are interested in individuals whose research addresses fundamental issues in neuroscience and who show significant potential for innovation, scholarship, and commitment to excellence in research and teaching.

Successful candidates will be expected to establish and maintain a high-profile research program attracting substantial extramural funding. The appointee will have access to state-of-the-art life science research support facilities and opportunities to interact with colleagues in the Institute for Complex Systems, Cognitive Neurology and Alzheimer's Disease Center, Center for Reproductive Science, Center for Sleep and Circadian Biology, Robert H. Lurie Comprehensive Cancer Center and an interdepartmental neuroscience graduate program with over 130 faculty.

Applicants will submit (in PDF format) a cover letter, a CV, and a description of research plans. For details on preparing the application, please visit [www.neurobiology.northwestern.edu](http://www.neurobiology.northwestern.edu). Please plan to request at least three letters of recommendation. Applications received by month date, year will be ensured full consideration. All other inquiries may be directed to **faculty search email address**.

*AA/EOE. Women and minority applicants are encouraged to apply.*

## Tenure-Track Faculty Position in Cancer Biology

The Virginia Tech Carilion Research Institute (VTCRI) in Roanoke, Virginia (<http://research.vtc.vt.edu/>) is recruiting a tenure-track/tenured faculty member in cancer biology. The position may be filled at the Assistant, Associate or full Professor level. The successful candidate will have a Ph.D. (or M.D./Ph.D. or D.V.M./Ph.D.), postdoctoral training experience and a record of significant accomplishment appropriate for rank. The position is a 12-month appointment with primary emphasis on research. Established investigators with strong innovative research programs and extramural funding are encouraged to apply although promising junior candidates will be considered. Start-up packages, new facilities, and support are highly competitive.

The VTCRI opened in the summer of 2010 and currently has 20 faculty research team leaders in structural biology, molecular and cellular neurobiology, molecular virology, tumor biology and genetics, cardiac and regenerative medicine. Current areas of focus in cancer biology include malignant brain tumors and breast cancer. The Institute has state-of-the-art facilities in molecular biology, optical imaging, high field cryo-electron microscopy, and magnetic resonance imaging, electrophysiology, computational and high capacity data analysis/storage and a new vivarium. During this period of major growth of the new institute, we are especially interested in colleagues who enjoy a highly collaborative environment and interacting with investigators from their own, as well as other disciplines including those working at molecular, cellular, systems, and computational levels with animal models of human disease and/or humans. Investigators using molecular genetic approaches to develop innovative approaches for identifying biomarkers, novel diagnostics and potential therapeutic targets are encouraged to apply.

The VTCRI is immediately adjacent to the Carilion Clinic and hospital and the new VTC School of Medicine where all medical students carry out four year research projects. The Institute has strong collaborative ties with the Virginia Bioinformatics Institute (VBI) and the School of Biomedical Engineering and Sciences (SBES) at Virginia Tech (VT), as well as with the VT Departments of Biological Sciences, Biochemistry, Physics, Psychology and the College of Veterinary Medicine. The Research Institute and Medical School are located in the picturesque Roanoke Valley midway between Washington, DC and Charlotte, NC.

Interested and competitive candidates should send a cover letter, their full CV, statement of research plans, and the names, full addresses and email addresses of three references to the attention of: Cancer faculty position-VTCRI at [secastle@vtc.vt.edu](mailto:secastle@vtc.vt.edu) before November 1, 2012, as well as posting these materials on the Virginia Tech jobsite at <https://listings.jobs.vt.edu/applicants/Central?quickFind=196038>. The three support letters should be addressed to **Michael J. Friedlander, Ph.D., Executive Director, VTCRI** and sent by email directly from the referees to Ms. Sarah Castle at [secastle@vtc.vt.edu](mailto:secastle@vtc.vt.edu) indicating Cancer Position.

*Virginia Tech is an Equal Opportunity/Affirmative Action Institution.*

University of Nebraska-Lincoln  
Institute of Agriculture and Natural Resources

## Hydrogeophysicist, Assistant Professor Institute of Agriculture and Natural Resources and the Robert B. Daugherty Water for Food Institute University of Nebraska

The Institute of Agriculture and Natural Resources at the University of Nebraska, with support from the Robert B. Daugherty Water for Food Institute (DWFI), is completing a cluster hire of faculty members to build on existing expertise in water for food at the university. In addition to this hydrogeophysicist position, two crop simulation modelers and an irrigation engineer have been hired, the search for a hydroinformaticist is close to completion, and a cropping systems agronomist will be hired. This team of faculty members will define and address compelling issues in the use of water in Nebraska's agriculture, especially those issues related to sustainable agricultural, ecological, and environmental systems that have global implications. Visit the WFI web site for more information on the program <http://waterforfood.nebraska.edu/>

This is a 12-month, tenure-track position at the assistant professor rank in the Institute of Agriculture and Natural Resources, with appointments in teaching (15%), research (50%), and extension (35%). The academic home could be in the School of Natural Resources, the Department of Agronomy and Horticulture, the Department of Biological Systems Engineering, the Department of Statistics, or a combination depending on the successful candidate's area of expertise. The successful candidate is expected to lead and coordinate a nationally-recognized research and education program in ground water characterization and management in agricultural regions with limited water supplies, and will be a member of a team using a systems approach to address managing watersheds to maintain economic and environmental well-being. To succeed in this role, a Ph.D. in hydrogeophysics, environmental geophysics, hydrogeology, or hydrology with geophysics experience, or a closely related field is required. A strong commitment to education and research; excellent communication skills; and the ability and desire to work cooperatively on multi-disciplinary projects are also required. Interest in water requirements of agricultural production systems is desirable.

To view the complete position details and make application for this position, go to the UNL Employment web site: <http://employment.unl.edu>, search requisition number **120760**. Complete the faculty academic administrative information form and attach a letter of application, curriculum vitae, and contact information for three professional references. Review of applications will begin on **November 1, 2012**, and continue until the position is filled or the search is closed.

*The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through Affirmative Action, Equal Opportunity, work-life balance, and dual careers.*

## Director of Research and Director of Policy Robert B. Daugherty Water for Food Institute University of Nebraska

The University of Nebraska (NU) is seeking two dynamic and creative leaders with vision to be the Director of Research and the Director of Policy in the Robert B. Daugherty Water for Food Institute (DWFI) at the University of Nebraska. The Director of Research will be responsible for leading and coordinating the research programs of the DWFI and for fostering the development of strong partnerships and an extensive knowledge base to develop innovative, effective solutions to the global challenge of securing more food with less water. The Director of Policy will be responsible for leading and coordinating the Institute's policy development and analysis activities and programs. Both positions are tenure-eligible, full-time appointments reporting to the Executive Director of the Water for Food Institute. Faculty rank and tenure will be available to candidates with a terminal degree and appropriate academic accomplishments. These positions are part of the Institute's senior management team which includes the Director of Policy, the Director of Research, the Associate Director, and the Director of the Nebraska Water Center. Additional information on the positions and the University of Nebraska can be found at: <http://waterforfood.nebraska.edu>.

Individuals interested in making application should access the web site: <http://employment.unl.edu>, search for requisition number 120421 (Director of Research) or requisition number 120175 (Director of Policy) and complete the faculty academic administrative information form. Attach a letter of application, a curriculum vitae, contact information (mailing address, phone number, and e-mail address, if available) for three professional references, and a vision statement for a research agenda (req. #120412) or vision statement for policy research and analysis (req. #120175) for the Daugherty Water for Food Institute (Other). Review of applications will begin **November 1, 2012**, and will continue until the positions are filled or the searches are closed.

*The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through Affirmative Action, Equal Opportunity, work-life balance, and dual careers.*





**EPPLEY INSTITUTE**  
FOR RESEARCH IN CANCER

Eppley Institute, home of the NCI-designated University of Nebraska Medical Center Eppley Cancer Center, seeks candidates for full-time tenure track faculty positions. Candidates are expected to develop an independent extramurally funded research program in basic/translational cancer research complementary to existing strengths and allied with the overall directions of the Center, participate in graduate-level teaching in the Cancer Research Graduate Program and/or other UNMC graduate programs and engage in institutional service based on their expertise.

Areas of particular interest include chemical biology with the objective of (a) identifying novel therapeutic agents using high throughput/systems biology, (b) targeted cancer therapies, (c) experimental cancer models and (d) biological evaluation of nanomedicine cancer therapies. Eppley currently has 30 tenured tenure-track faculty and the Cancer Center has over 100 primary members that focus on externally funded research.

Candidates must have a Ph.D. and/or a M.D. degree and postdoctoral research experience. Faculty rank and compensation DOQ. Start date is July 1, 2013. Applicants must apply online at <http://jobs.unmc.edu/postings/13764> and attach CV and three letters of reference. Additional information can be found at <http://www.unmc.edu/cancercenter/>.

*EEO/AA individuals from diverse backgrounds are encouraged to apply.*



## FACULTY POSITION IN CELL BIOLOGY BIOLOGY DEPARTMENT BOSTON COLLEGE

The Boston College Biology Department seeks outstanding candidates for a tenure-track faculty position at the level of **ASSISTANT PROFESSOR (or ASSOCIATE PROFESSOR)**. Boston College provides competitive start-up funds and research space, with the expectation that the successful candidate will establish, or bring to the university, a vigorous, externally-funded research program. The successful applicant will have access to well-equipped animal facilities, core laboratories with state of the art instrumentation for fluorescence microscopy and flow cytometry, and substantial computational resources. We seek a cell biologist studying a basic mechanism in cell biology using a model system and/or one who uses or is developing new technologies. Special consideration will be given to candidates whose research program complements current faculty interests in cell biology (microbes, pathogens, microbe-host interactions, cell division, genetics, genomics, and bioinformatics: (see <http://www.bc.edu/biology> for profiles of current faculty research programs)). In addition, the successful candidate will be expected to train graduate students and participate in the undergraduate teaching mission of the Biology Department. This appointment will begin on or after July 1, 2013.

Applicants should submit a single file, containing a curriculum vitae and a statement of present and future research plans (please use surname as filename). Applicants should also arrange to have three letters of reference submitted separately. All documents should be submitted as .pdf files to [biosearch@bc.edu](mailto:biosearch@bc.edu)

Applications received by **November 15, 2012** are assured of full consideration. Review of applications will continue until the position is filled.

*Boston College is an Affirmative Action, Equal Opportunity Employer committed to improving diversity.*



**UNITED NATIONS  
UNIVERSITY**

The UNU is the academic arm of the United Nations system. Its mission is to contribute through collaborative research and postgraduate education, dissemination of knowledge and advisory services, to efforts to resolve the pressing global problems of human survival, development and welfare that are the concern of the United Nations, its Peoples and Member States. The University functions as a think tank for the United Nations system and for UN Member States providing knowledge-based policy advice.

### DIRECTOR (D-1 LEVEL) UNU-INTERNATIONAL INSTITUTE FOR GLOBAL HEALTH (UNU-IIGH)

(DUTY STATION: KUALA LUMPUR, MALAYSIA)

The UNU-IIGH Director serves as the Chief Academic and Administrative Officer of the Institute and has overall responsibility for the direction, organization, administration and programmes of the Institute under the direction of the Rector of the UNU.

**Qualifications:** A Doctorate in the field of Health Systems and Policies, Public Health, Epidemiology or other health-related discipline. Strong research background and publications in areas related to emerging problems in public health such as non-communicable diseases, including global mental health; expertise related to tele-health and e-health issues highly desirable. A proven record of effective leadership and management experience at a Senior level in Academic or Research Institutions.

**Experience:** Candidates should possess excellent communication skills with fluency in English and at least one other official language of the United Nations.

Applications from suitably qualified women candidates are particularly encouraged.

**CLOSING DATE: 31 OCTOBER 2012**

Please visit <http://unu.edu/about/hr> for the full job description, requirements and application procedures. Application by email is highly encouraged.

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## PRIZES



### Launch of MERAC Prizes for young European astronomers

FONDATION MERAC is a non-profit foundation started in 2012 with headquarters in Switzerland to recognize and support young European astronomers. There are yearly three MERAC Prizes awarded by the European Astronomical Society (EAS). The prizes of EUR 20'000.- are for each of the three categories: Theoretical Astrophysics, Observational Astrophysics, and New Technologies (Instrumental/ Computational).

The EAS Council invites EAS members to nominate suitable candidates for the MERAC Prizes of 2013. This being an uneven year, the three prizes will be Best Early Career Researcher Prizes.

**Sunday, September 30, 2012**

is the nominal deadline for nominations at:

[http://eas.unige.ch/merac\\_prizes.jsp](http://eas.unige.ch/merac_prizes.jsp)

## POSITIONS OPEN



**CASE WESTERN RESERVE UNIVERSITY** EST. 1826

The Department of Otolaryngology Head & Neck Surgery, Case Western Reserve University (<http://casemed.case.edu/otolaryngology/research.html>) is seeking applications for an assistant professor (basic science) tenure-track faculty position. We are interested in applicants whose research relates to inner ear development, function, and/or disease. A successful candidate will be a member of the vibrant and well-funded Hearing Research Program within the department. CWRU provides an excellent interdisciplinary and collaborative intellectual environment.

The candidate is expected to develop a strong program of research, be actively involved in resident research activities and participate in teaching activities appropriate to his/her area of interest. Close collaboration with academic clinicians is encouraged. The successful applicant will have the opportunity to collaborate with faculty members in other Departments at CWRU including The Departments of Genetics and Genome Sciences, Neurosciences, Pharmacology, and Biomedical Engineering in addition to The Center for Proteomics and Bioinformatics and The Center for Stem Cell and Regenerative Medicine. Successful candidates will be encouraged to seek secondary faculty appointments in The Departments of Genetics and Genome Sciences or Neurosciences. Competitive startup package and laboratory space are available.

Candidates should hold an M.D. or Ph.D., postdoctoral research experience, a strong publication record, and the potential to secure extramural funding. Send a statement of research and teaching interests, curriculum vitae, and the names, addresses, and phone numbers of three to five references to Dr. Alagramam. Evaluation of the applications will begin **October 1, 2012**. Applications will be accepted until the position is filled. In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. Women, veterans, members of underrepresented minority groups, and individuals with disabilities are encouraged to apply.

Case Western Reserve University provides reasonable accommodations to applicants with disabilities. Applicants requiring a reasonable accommodation for any part of the application and hiring process should contact the Office of Inclusion, Diversity and Equal Opportunity at 216-368-8877 to request a reasonable accommodation. Determinations as to granting reasonable accommodations for any applicant will be made on a case-by-case basis.

**Kumar N. Alagramam, Ph.D. – Chair of the Search Committee**  
**The Maniglia Chair for Research and Education**  
**Associate Professor and Director of Research**  
**Otolaryngology Head & Neck Surgery**  
**Case Western Reserve University**  
**Cleveland, OH**  
**Email: [kna3@case.edu](mailto:kna3@case.edu)**

## POSITIONS OPEN

# YaleNUS College

### Environmental Studies at Yale-NUS College

The newly established Yale-NUS College in Singapore, a collaboration between the National University of Singapore (NUS) and Yale University, is seeking faculty of all ranks in the broad area of Environmental Studies. We are interested in teacher-scholars who work at the intersection of the natural and social sciences, including but not limited to environmental economics/risk assessment, global change and society, environmental law and policy, and natural resource sustainability in Asia, other regions and worldwide.

We seek outstanding candidates for the inaugural faculty who have a particular interest in undergraduate teaching in a residential setting and are committed to innovative pedagogy. Candidates with enthusiasm for contributing to an interdisciplinary common curriculum in the liberal arts are particularly welcome. Faculty members are expected to maintain a strong research focus, with particular strength in mentoring undergraduates in research experiences. Startup funds, and continuing research funds on a competitive basis, are available through the College.

Environmental Studies is one of three interdisciplinary majors (Urban Studies and Global Affairs are the others) that draw from the natural sciences, arts and humanities. The College will not have disciplinary departments, but joint appointments with NUS departments can be arranged in cases where the involvement of Yale-NUS faculty with research facilities, graduate students, and other activities at NUS would be mutually beneficial. Salary, benefits and leave policies will be competitive at an international level.

The College values diversity and is committed to equality of opportunity. All particulars of the application process and links to the application site and to other information about Yale-NUS can be found at [www.yale-nus.edu.sg/prospective-faculty.html](http://www.yale-nus.edu.sg/prospective-faculty.html). Review of applications begins on **November 2, 2012** and will continue until positions are filled.

## ANNOUNCEMENTS



**SOUTH DAKOTA SCHOOL OF MINES AND TECHNOLOGY CONGRATULATES THE 2012 MINES MEDALIST**



### DR. DIANA WALL

One of the world's foremost experts on biodiversity, Dr. Wall's groundbreaking research has led her from the Antarctic Dry Valleys to sub-Saharan Africa. She is Director of the School of Global Environmental Sustainability at Colorado State University, and is researching habitat diversity and soils.

The South Dakota School of Mines and Technology launched the Mines Medal Award Program in 2009 to honor engineers, scientists, and researchers who have demonstrated exceptional leadership and innovation. The award also highlights the significant role these individuals play in our society, helping to ensure our nation's global preeminence in engineering and science.  
[www.sdsmt.edu](http://www.sdsmt.edu)





## POSITIONS OPEN

### OPEN POSITION: LECTURER with Potential for Security of Employment The School of Biological Sciences University of California Irvine

Applications are invited for a full-time academic year Lecturer with Potential for Security of Employment to coordinate undergraduate and Master's programs in Biological Sciences Education. Salary will be commensurate with education and experience. This individual will be qualified to teach in both the Biological Science Education programs and the undergraduate Biological Sciences programs. The successful applicant must have a Ph.D. in biology, or a related scientific discipline, and a demonstrated commitment to K-12 science education, such as experience teaching science to high school students. Preference will be given to applicants who have the ability to write successful grant applications and/or who have coordinated other academic programs. Please submit curriculum vitae, a description of both your background in education and your academic experiences, as well as three letters of recommendation. Review of applications will begin on December 1, 2012, but the position will remain open until filled. Applicants should use the following online recruitment URL to submit the requested material **website: <https://recruit.ap.uci.edu/apply/JPF01808>**.

*University of California Irvine (UCI) is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.*

### VERTEBRATE PHYSIOLOGIST Dickinson College

The Biology Department at Dickinson College seeks a vertebrate physiologist to fill a new tenure-track **ASSISTANT PROFESSOR** position beginning July 1, 2013. A candidate with research and teaching experience in the fields of endocrinology, pharmacology, and/or toxicology is desired. Teaching responsibilities will include introductory biology, as well as upper-level courses in physiology and the candidate's area of expertise, in a department that supports major programs in Biology, Biochemistry and Molecular Biology, and Neuroscience. The successful applicant will have access to startup and institutional research funds, laboratory, and office space in the newly completed Rector science complex, and will be expected to develop a vigorous research program involving undergraduates. A Ph.D. is required; postdoctoral experience is preferred. Review of applications will begin on October 8, 2012. To apply, send a letter of application, curriculum vitae, statements of teaching philosophy and research interests, and three letters of reference to **website: <https://jobs.dickinson.edu>**. Located in south central Pennsylvania, Dickinson (enrollment of 2,300) is a highly selective national liberal arts college with an emphasis on innovative science teaching and student/faculty research.

### OPPORTUNITY DEVELOPMENT MANAGER-SOUTH CENTER U.S.

Opportunity for Opportunity Development Manager at The Jackson Laboratory, located in Bar Harbor, Maine, covering South Central U.S. (Texas, Oklahoma, Louisiana, Alabama, Colorado, and Utah) Field Sales. Duties include identifying, negotiating and managing strategic relationships with biomedical, academic and pharmaceutical partners with regular, direct, personal contact to present and prospective customers; overall responsibility for ensuring revenue and management objectives are met and exceeded for assigned territory. Position requires a Master's degree or higher in Life or Animal Sciences field, or Bachelor's degree in same plus five years or more progressive direct biomedical or animal science sales experience including documented, successful field management experience or equivalent leadership experience. Position requires extensive travel throughout assigned territory, and residence near major airport in assigned territory. Applicants should apply online at **website: <http://www.jax.org>** with a cover letter and resume to **job requisition #3434**.

## POSITIONS OPEN

### FACULTY POSITION

#### Assistant Professor in Eukaryotic Cell Biology

The Department of Biological Sciences at Wellesley College invites applications for a tenure-track faculty position at the rank of Assistant Professor to start in July 2013. We seek a broadly trained cell biologist who is strongly committed to excellence in both teaching and research in an undergraduate liberal arts college environment. The position is open to any area of eukaryotic cell biology; however, we are particularly interested in candidates with expertise in systems biology. In addition to teaching in our cellular biology core curriculum, successful candidates would be expected to offer advanced courses in their specialty, and to develop an active research program that involves undergraduates. A Ph.D. and postdoctoral experience are required. Applications should include a cover letter, curriculum vitae, statements of teaching and research interests, and three letters of recommendation (the online application will request name and e-mail address in order for recommenders or dossier services to submit letters directly). Materials should be submitted by visiting our application **website: <https://career.wellesley.edu>**. If circumstances make it impossible to submit materials online, please send to **e-mail: [working@wellesley.edu](mailto:working@wellesley.edu)**. The deadline for receipt of all application materials is October 22, 2012.

*Wellesley College is an Affirmative Action/Equal Opportunity Employer, and we are committed to increasing the diversity of the college community and the curriculum. Candidates who believe they can contribute to that goal are encouraged to apply.*

### TENURE-TRACK POSITION Department of Chemical Engineering and Materials Science University of California, Davis

Applications are invited for a faculty position at the **ASSISTANT PROFESSOR** level in chemical engineering. All areas of expertise will be considered, and candidates with an interest in the area of catalysis are especially encouraged to apply. The candidate should have a strong research record with the potential and commitment to become a leader in the field. Commitment to undergraduate and graduate education is essential. A Ph.D. in chemical engineering or a related discipline is required. Consult **website: <http://chms.engineering.ucdavis.edu/>** for our on-line application procedure and requirements. The position is open until filled; but to assure full consideration, applications should be submitted no later than October 19, 2012, for a start date of July 1, 2013.

*UC Davis is an Affirmative Action/Equal Opportunity Employer, and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, individuals with disabilities, and veterans.*

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Professor William E. Rees and Dr. Mathis Wackernagel are advocates for the "Ecological Footprint," a unique index linking limited ecosystem services with human resource consumption. The graphic power of the Footprint metaphor in many languages has helped make the Footprint the world's best-known and most-used sustainability metric. Their study has significantly influenced society, prompting wide reflection on excess consumption.



**Dr. Thomas E. Lovejoy**  
(USA)

Dr. Thomas E. Lovejoy was the first scientist to academically clarify how humans are causing habitat fragmentation and pushing biological diversity toward crisis. He has influenced numerous academics and societies and has helped to protect the natural environment based on biological diversity, which is now a mainstream concept.



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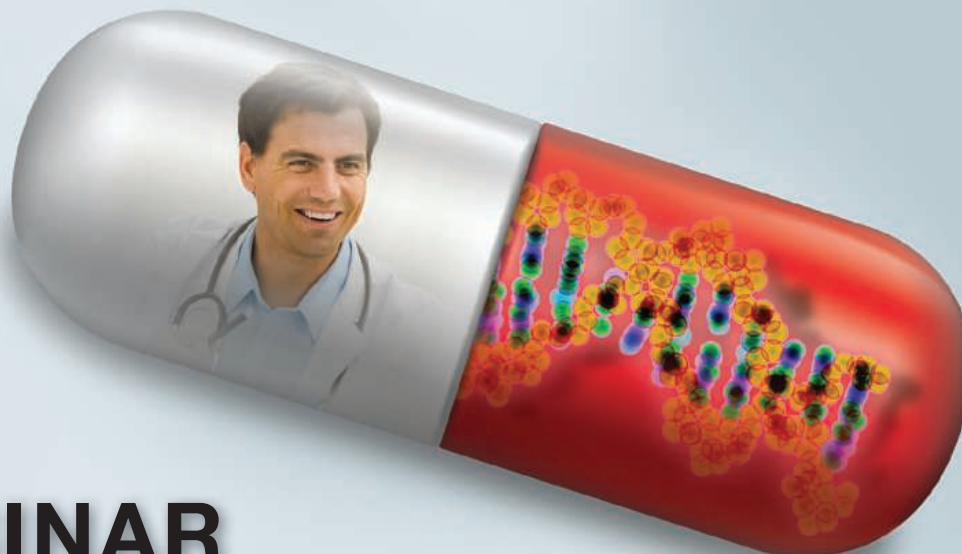
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# WEBINAR

## Translating Genetic Biomarkers to the Clinic

The Promise and Pitfalls of Developing Robust, Reliable Signatures

### Participants

**Timothy J. Yeatman, M.D.**  
Moffitt Cancer Center  
Tampa, FL

**Henk Viëtor, M.D., Ph.D.**  
Skyline Diagnostics  
Rotterdam,  
The Netherlands

**Wednesday, October 3, 2012**

**12 noon ET, 9 a.m. PT, 4 p.m. GMT, 5 p.m. UK**

As our understanding of the human genome expands, so does the desire to translate this knowledge into effective clinical tools. With a growing number of complex genomic tests for biomarker signatures becoming commercially available, the promise of personalized medicine is fast becoming a reality. Although researchers and clinicians are continuously striving to enable quicker and more individualized treatment decisions, the development of these tests is a long road, with discovery and validation of genomic biomarker signatures just the start. Our expert panel will discuss the practicalities of developing a test from signature discovery and validation all the way to clinic.

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